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II. TRANSMISSION OF SOUND ENERGY AND THICKNESS OF PLATE TRANSMITTER AT NORMAL INCIDENCE—ULTRASONIC METHOD[1]

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Abstract

Another and more direct experimental method for determining the reflection from and transmission through parallel-faced partitions of sound energy of short wave-length was studied. Measurements were taken of the energy transmitted through obstructing plates by means of a torsion pendulum placed in the "ultrasonic shadow", the incidence of the energy being normal. The theoretical and experimental results obtained in previous researches by taking reflection measurements only were confirmed, and again it was shown necessary to employ the bulk modulus of elasticity for computations of velocity instead of Young's.

Introduction

In a paper published recently (3) Boyle and Froman described experimental investigations on the problem of reflection of ultrasonic waves, incident normally, and travelling in water, from plane parallel-faced partitions of thickness comparable with a wave-length. In the experiments metal vanes of torsion pendula, by which the quantitative measurements were taken, themselves acted as reflectors of the waves, and thus were made to yield the desired information concerning the reflection. The conclusion was confirmed that reflection was a maximum and transmission a minimum when the thickness of the reflector was an odd number multiple of a quarter wave-length, but reflection was minimum and transmission maximum when the thickness was an integral number multiple of a half-wave-length. In general, the principal suggestions of a mathematical solution of the problem outlined by Rayleigh (6) were well borne out, provided that the bulk modulus of elasticity was used to compute the velocity of propagation rather than Young's. The mathematical relations expressing the reflecting powers in this problem are given in the previous paper.

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The point of the present work is to show that the same experimental results may be arrived at by a more direct, though not easier, experimental method. Plane parallel-faced plates of different thicknesses were placed perpendicularly to the path of the ultrasonic beam, and the energy transmitted through a plate was measured by a torsion pendulum placed in the "ultrasonic shadow" behind it. A preliminary notice of this method was made by one of us (2), but it is only now that an opportunity is presented to describe the experiments fully.

Experimental Details

The ultrasonic beam was generated in the usual manner by exciting a common type of quartz piezo-electric generator from a source of high frequency electric oscillations, viz., electron valves. The generator was placed at one end of a large wooden tank, 15 ft. long, 5 ft. wide, 3.5 ft. high, containing water so that it projected its ultrasonic beam down the middle of the tank. Wooden baffling screens set at an angle of about 45° to the end of the tank opposite the generator served to dissipate the energy reaching them in multiple reflection, and thus avoided undue disturbances at the measuring instrument, near the middle of the tank, by reflection from the end. The measuring torsion pendulum was of the single "air-vane" type (2), with the usual mountings and torsion head. All the apparatus, with the single exception of the transmitting plate and its mountings, has been described before (1, 4). It should be mentioned here that in 1922, Boyle, assisted by J. F. Lehmann, carried out a few preliminary experiments on the transmission of ultrasonic energy through a plate by the same method. These experiments were of an approximate nature only and had to be discontinued at the time; but since they were the forerunner of the present investigation it was thought well to include their results in this report.

Older Experiments

A light wooden framework, annular in shape, supported the experimental plate in the large water tank. This plate was 12 in. square and served as a shutter for an aperture in the wooden frame; it opened or closed the aperture by its motion in a groove cut in the framework. The diameter of the aperture was just less than 12 in., and about equal to the diameter of the ultrasonic beam at the point. When the framework was supported in position and the aperture was open, the ultrasonic beam could pass centrally through it perpendicularly to its plane. Comparative observations of energy intensity were taken by means of a torsion pendulum (in this case a double-vane metal type) suspended in the axis of the beam, on the side of the shutter remote from the generator and not far from the aperture. With the aperture open the reading of the pendulum gave a relative measurement of the incident energy, when it was closed by lowering the shutter (experimental plate), the reading gave a measurement of the energy transmitted by the plate, since all other experimental conditions were kept the same. Hence the ratio of

transmitted to incident energy, at the frequency of experiment, could be easily ascertained. A few results were collected for various materials at frequencies ranging from 64,000 to 359,000 cycles per second[1].

The procedure of an experiment was to measure the energy emitted through the aperture, with and without the shutter, over a range of frequencies which included *that particular frequency at which it was anticipated the plate would be one half-wave-length thick.* Curves showing the manner in which the transmission energy ratio varied with the frequency were plotted; from which it could be ascertained at what frequency the transmission was a maximum. The results of these older experiments are collected in Table I.

TABLE I

RESULTS OF OLDER EXPERIMENTS

Material of plate	Thickness of plate, in cm.	Temperature, in ° C.	Frequency for maximum transmission, in cycles per sec.	Wave-length in material	Computed velocity, in cm. per sec.
Steel	1.55	13.2	185,000	3.10	5.73×10^5
	1.55	13.2	*359,000	3.10	5.56×10^5
	2.15	13.9	129,900	4.30	5.59×10^5
	2.15	14.7	132,000	4.30	5.68×10^5
	2.15	13.2	*263,000	4.30	5.65×10^5
Lead	0.814	11.0	140,700	1.63	2.29×10^5
	0.814	12.3	140,000	1.63	2.28×10^5
Glass	0.992	9.7	273,000	1.98	5.42×10^5
Marble	2.56	9.7	64,000	5.12	3.28×10^5
Maple wood (along fibre)	2.66	15.3	79,500	5.32	4.23×10^5

**At these frequencies the observations correspond to the second transmission maxima. The thickness of plate must then have been TWO half-wave-lengths instead of ONE.*

It may be remarked here that in the above experiments on the *steel* plates, when the plate was *in exact resonance* (i.e., at its half-wave-length thickness) with the frequency of the impinging waves, there seemed to be, sometimes, judging from the behavior of the measuring pendulum, a suggestion of an increased energy intensity along the axis of the beam at a distance from the plate. This is not impossible for, theoretically, the plate at resonance could act as a tuned resonator in an imposed sound field. It may be possible at a later date to undertake a special research by this method to investigate the point experimentally.

[1] *In these experiments there must have been some reflection of the waves from the wooden framework supporting the plate; these could interfere with the incident beam and thus mar the experiment to a slight extent.*

More Recent Experiments

The ultrasonic generator employed was one of the double steel plate (2-quarter-wave plate) type with 15.3 cm. diameter of radiating face. It was placed in the water tank about 160 cm. away from the experimental plate, in such a manner as to project its beam horizontally down the middle. The normal high frequency voltage on the generator during the experiments was 2,000 volts, and it was kept as nearly constant as possible. If there were slight divergencies from this voltage at the time of an observation corrections were made in the pendulum readings by the square law.

Instead of using a framework to support the plate in the ultrasonic beam, as in the older experiments, a better and easier method was employed of suspending the plate normally to the beam by two thin wires hanging from top supports. In this way there were no interfering reflections from any supporting framework. The measuring pendulum was placed in the "ultrasonic shadow" behind the plate, a few centimeters distant from it and on the axis of the beam. This pendulum was of the single air-vane type, 1.5 cm. in diameter, with a phosphor-bronze strip suspension 0.0038 cm. wide and 67 cm. in length from the torsion head.

One material only was used for the experimental plates, viz., type-metal, which was chosen because its "mass of a wave-length" (i.e., the product of wave-length and density) was less than that of most available materials; on this account the thickness and mass of the plates used were not inconveniently great (3). In addition this metal could be quickly and easily cast and machined when plates of a special thickness were required.

In this type of experiment it must be remembered that an *increase* in transmitted energy is indicated by a *rise* of pendulum deflection, and not by a fall as in the reflection method described previously (3). The procedure of an experiment was to maintain as constant as possible the experimental frequency and intensity of emission, and take a series of readings of pendulum deflections for plates of different thicknesses. On occasions a standard plate was inserted and the energy transmitted through it measured, in order to ensure the constancy of emission from the generator. If there were any slight variations in emission the deflections were numerically referred to that of the standard, thus making all the measurements accurately comparative. Corrected transmission ratios so obtained were plotted against the thickness of the transmitting plate. All the work described was carried out at ordinary temperature which varied from 17 to 19° C.

A first and preliminary series of experiments was carried out at the fixed frequency of 308,000 cycles per sec. with plates varying from 0.199 to 1.407 cm. in thickness. The observations are collected in Table II (a), and the corresponding transmission ratios are plotted in Fig. 1. There were two series of plates here used, the second considerably thicker than the first. It can be seen at a glance that there must have been a transmission maximum at a plate thickness approximating 0.4 cm. and another at a thickness near 1.2 cm.

Evidently the first thickness corresponded to about *one* half-wave-length and the second to about *three*. The wave-length at this frequency in the type-metal therefore should be about 0.8 cm.

Another series of experiments was then performed to cover the range of thicknesses which included *two* half-wave-lengths. The observations are collected in Table II (b), and the corresponding transmission ratios are plotted in Fig. 1. In this series 306,500 cycles per sec. was as close as it was possible to adjust the frequency to the former value. The results, as can be seen from Fig. 1, showed that the wave-length, as anticipated, was about 0.8 cm.

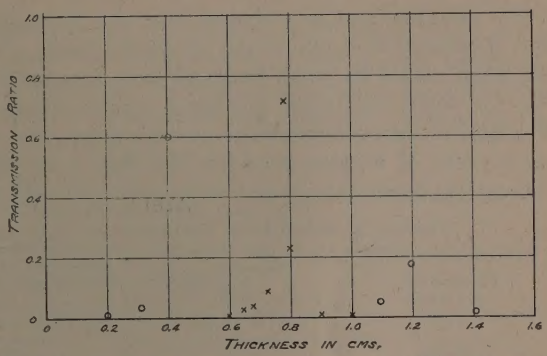

FIG. 1. *Relation of transmission ratios to thickness of plate. Circles denote values obtained with a frequency of 308,000 cycles per sec; crosses, those obtained with 306,500 cycles per sec.*

TABLE II

THICKNESS OF PLATE AND CORRESPONDING DEFLECTIONS*

Plate thickness, in cm.	Pendulum deflections		Ratio
	With plate	Without plate	
(a) With constant frequency of 308,000 cycles per sec.			
0.199	2	200	0.010
0.309	7	198	0.035
0.403	131	198	0.060
1.094	10	198	0.050
1.198	34.5	192	0.180
1.407	3.5	198	0.018
(b) With constant frequency of 306,500 cycles per sec.			
0.600	0.5	196	0.0025
0.646	4.3	196	0.022
0.677	7.0	196	0.036
0.727	10.5	196	0.084
0.782	140.0	196	0.714
0.801	44.5	196	0.230
0.902	1.9	196	0.009
1.002	1.4	196	0.007

**With type-metal plates*

By changing the method of experiment the frequency to make the thickness of a given plate exactly equal to an integral number of half-wave-lengths can be directly determined. Instead of fixing the frequency and changing the plate thickness, an experimental plate may be chosen of thickness near the expected half-wave-length and the frequency be carefully adjusted until the transmission, as shown by an indicating pendulum, is a maximum.

For example, and as a typical case, the plate 0.403 cm. thick was chosen and suspended normally in the ultrasonic beam. The frequency of excitation was then varied by small intervals over a range of frequencies including the one for which this plate thickness was expected to be a half-wave-length. Readings of pendulum deflections were taken, and also the deflections over the same range of frequencies with the plate removed. The observations are collected in Table III

TABLE III

PENDULUM DEFLECTIONS CORRESPONDING TO VARIOUS FREQUENCIES*

Frequency, in cycles per sec.	Pendulum deflections		Transmission ratio (c)— from Curves
	(a) With plate	(b) Without plate	
277,000		7.1°	
283,500	5.0°		0.45
285,000		13.0	0.53
288,000		18.5	0.71
288,500	14.0		0.70
290,500	20.0		0.77
293,000	34.0		0.94
293,200		37.0	0.92
295,000			0.85
297,000	47.0	65.0	0.72
299,500	76.0		0.69
300,300		125.0	0.61

**With type-metal place, 0.403 cm. thick*

NOTE:—*In this type of experiment it is best to use an ultrasonic generator which is well damped, and therefore with characteristic energy emission curve not made up of sharp peaks and hollows. In this respect the generator used here was not particularly suitable, but it was the only one that could be used at the time.*

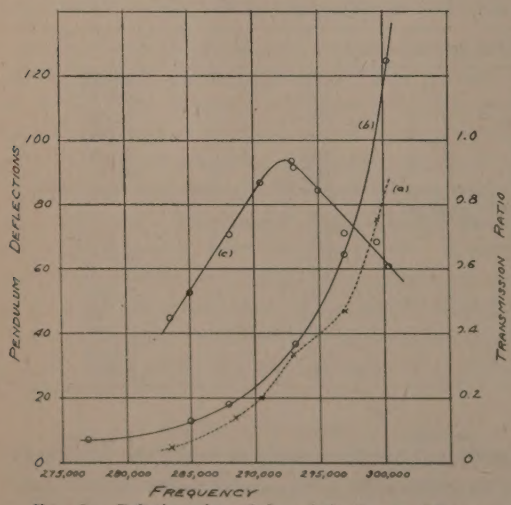

FIG. 2. *Relation of pendulum deflections and transmission ratio to frequency.*

From this experiment *the actual pendulum deflections* were plotted against frequency, for the curves depend greatly on the "characteristic" energy emission curve of the generator. Curve (a), Fig. 2, represents the transmitted energy with plate intervening, curve (b) the energy unobstructed by the plate. The transmission ratios for the various frequencies are plotted in curve (c). An inspection of the curve (c) of Fig. 2 shows that the transmission ratio was a maximum for this plate 0.403 cm. thick when the frequency was 292,300 cycles per sec. Therefore the velocity of the waves in the material, given by twice the product of these figures, is 2.35×10^5 cm. per second.

Four such series of experiments were carried out with plates equal to one, two, and three half-wave-lengths, approximately. The exactly determined frequencies for maximum transmission gave computed results for the velocity in remarkably good agreement. These results are collected in Table IV.

TABLE IV

VELOCITY IN PLATE FROM THE FREQUENCY OF MAXIMUM TRANSMISSION

Plate thickness, in cm.	Frequency for maximum transmission, in cycles per sec.	Number of half-wave-lengths in plate	Computed velocity, in cm. per sec.
0.403	293,000	1	2.35×10^5
0.801	296,000	2	2.37×10^5
1.198	296,000	3	2.37×10^5
0.672	527,500	3	2.36×10^5
			Average 2.36×10^5

With the correct value of velocity it is now possible to compute the transmission ratio for any thickness of plate (neglecting absorption), and thus completing the plotting of Fig. 1, to construct the full curve.

But it was considered desirable to confirm the determination of velocity as just described by resorting to the reflection method as described in the previous paper (3). Torsion pendula were made of the same material, i.e., type-metal, as the plates already used, and experiments were carried out in exactly the same detail as did Boyle and Froman (3). The pendulum vanes were all of the same diameter viz., 2.5 cm. and varied from 0.228 cm. to 0.578 cm. in thickness. They were suspended about 160 cm. from the generator at the centre of the beam, by a phosphor-bronze wire 82 cm. long and 0.0025 cm. in diameter. Two series of readings were taken, one at the frequency of 306,500 and the other at 529,500 cycles per sec. Pendulum readings were taken with the different thicknesses of pendulum vane, and plotted against the thickness. It must be remembered that in this case a *decrease* of pendulum

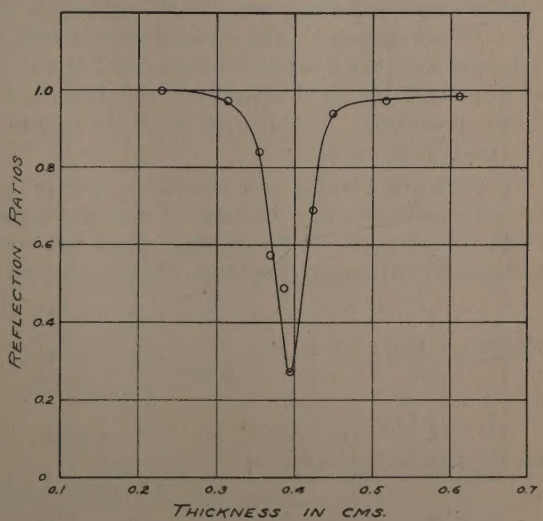

FIG. 3. *Relation of ratio of reflected to incident energy to thickness of plate reflector (Type-metal pendulum, frequency 306,500 cycles per sec.).*

reading indicates an increase of transmission through the vane. The observations in both series are collected in Table V. As an example Fig. 3 shows the results of the series at the frequency 306,500 cycles per sec.

TABLE V

THICKNESS OF PENDULUM AND CORRESPONSING DEFLECTION

Frequency 306,500 cycles per sec.			Frequency 529,500 cycles per sec.		
Vane thickness, in cm.	Pendulum deflections	Reflection ratio	Vane thickness, in cm.	Pendulum deflections	Reflection ratio
0.228	40.0°	1.00	0.131	38.7°	1.00
0.314	39.0	0.975	0.197	26.5	0.94
0.354	33.5	0.84	0.211	26.3	0.68
0.369	23.0	0.575	0.221	18.2	0.47
0.387	19.5	0.49	0.228	13.0	0.34
0.395	11.0	0.275	0.233	18.0	0.405
0.424	27.5	0.69	0.239	27.1	0.70
0.449	37.5	0.94	0.252	34.3	0.89
0.518	39.0	0.975			
0.611	39.5	0.986			

The curve of Fig. 3 shows that the half-wave-length for the frequency 306,500 cycles per sec. must be very near 0.395 cm. By the more precise method of using this pendulum to measure deflections and varying the frequency carefully until the reflection ratio was a maximum, under otherwise identical conditions of experiment, it was found that the frequency at which 0.395 cm. was the exact half-wave was 303,800 cycles per sec. Hence the velocity is 2.40×10^5 cm. per sec. The series of experiments at the frequency of 529,500 cycles per sec. gave a value of velocity of 2.38×10^5 cm. per sec. These values are in remarkably good agreement with those given by the plate method as already described. The average of all the results obtained by both methods is 2.37×10^5 cm. per sec.

Knowing this velocity, it is possible to deduce reflection ratio and transmission ratio curves for any thickness of obstructing partition expressed as a ratio of the wave-length. The frequency does not enter into the computations. As shown by Boyle and Rawlinson (5) the reflected energy, compared with the incident, is given by

$$R = \frac{\left(\frac{V\rho}{V_1\rho_1} - \frac{V_1\rho_1}{V\rho}\right)^2}{4\cot^2\frac{2\pi l}{\lambda_1} + \left(\frac{V\rho}{V_1\rho_1} + \frac{V_1\rho_1}{V\rho}\right)^2},$$

and the transmitted by

$$E = \frac{4\operatorname{cosec}^2\frac{2\pi l}{\lambda_1}}{4\cot^2\frac{2\pi l}{\lambda_1} + \left(\frac{V\rho}{V_1\rho_1} + \frac{V_1\rho_1}{V\rho}\right)^2}.$$

Since we assume that, practically, absorption can be neglected in the first few half-wave-lengths—these two expressions add up to unity, since $R+E=1$.

Hence, taking the velocity in type-metal as 2.37×10^5 cm. per sec., and knowing the density, which was specially determined and found to be 10.02, the transmission ratios (E) for any fractional thickness of a wave-length may be computed as in Table VI.

TABLE VI

THEORETICAL TRANSMISSION FOR VARIOUS THICKNESS RATIOS*

$\frac{l}{\lambda_1}$	$4 \cot^2 \frac{2\pi l}{\lambda_1}$	E	$\frac{l}{\lambda_1}$	$4 \cot^2 \frac{2\pi l}{\lambda_1}$	E
0.00	∞	1.00	0.25	0.000	0.014
0.01	1011	0.80	0.30	0.422	0.017
0.015	450	0.64	0.34	1.61	0.021
0.02	251	0.50	0.40	7.62	0.042
0.025	159	0.39	0.45	37.9	0.14
0.03	110	0.31	0.47	110	0.31
0.05	37.9	0.14	0.475	159	0.39
0.10	7.62	0.042	0.48	251	0.50
0.16	1.61	0.021	0.485	450	0.64
0.20	0.422	0.017	0.49	1011	0.80
0.25	0.000	0.014	0.50	∞	1.000

**Velocity of sound in water, $V = 1.48 \times 10^5$ cm. per sec.*
Velocity of sound in type-metal, $V_1 = 2.37 \times 10^5$ cm. per sec.
Density of water, 1.00; density of type-metal, 10.0 gm. per cm³.
NOTE:—*The theoretical curve repeats itself in each half-wave-length since absorption is neglected.*

The theoretical values of the transmission ratio E are plotted against fractional thicknesses of a wave-length in Fig. 4. The points marked with a small circle are the experimental values taken from the observations given in Table II and estimated on the value of 2.37×10^5 cm. per sec. for the velocity as here experimentally determined. The agreement is remarkably good.

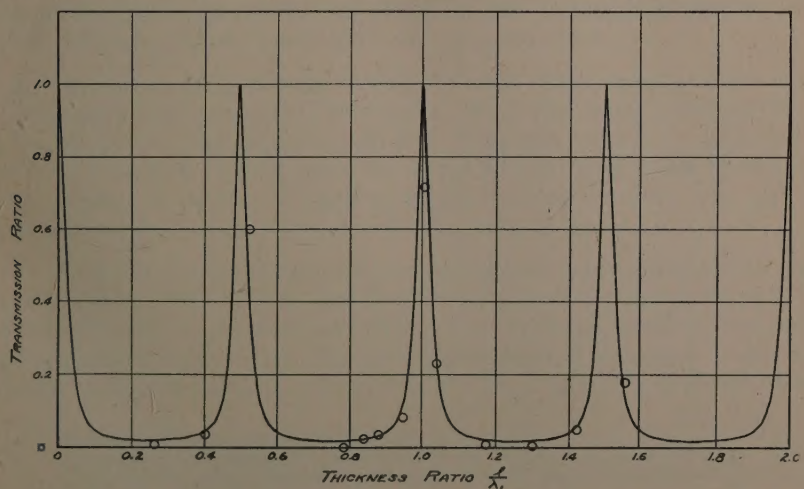

FIG. 4. *Relation of transmission ratio and the ratio of thickness of plate to wave-length. Plotted from theoretical values and experimental results (circles). Type-metal plate.*

In this, as in previous work on the same problem, the velocity in thin plates or partitions comes out higher than would be appropriate in the case of rods or bars, where Young's modulus would apply in the estimation of the velocity from the physical constants of the material. In a rod of this type-metal, using a method of ultrasonic longitudinal vibrations incidental to other work (and not yet published), it was found that the velocity was 1.71×10^5 cm. per sec. From this value it is calculated that *Young's Modulus* for this metal at ordinary temperature is 2.93×10^{11} dynes per cm^2, whereas from the value of velocity 2.37×10^5 cm. per sec., as found for plates by the method of this paper, the *bulk modulus* is 5.63×10^{11} dynes per cm^2.

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DURATION OF META-STABLE STATES[1]

By J. M. Anderson[2]

Abstract

The half-value time of absorption from meta-stable electronic atomic states is calculated on the hypothesis that the whole decay is due to collision phenomena. Definite assumptions, which include the atomic energy level scheme, are made as to the efficiency of collisions and the interaction of two meta-stable states in these processes. The assumption adopted as the only one producing an approximation to experimental facts shows that the probability of an exchange is dependent on the *absolute value* of the energy difference between the states concerned. The results calculated from the final formula, for helium, neon and argon at normal and very low temperatures depend on one arbitrary constant, which is the same for all states and gases considered, and show fair agreement with any experimental values so far published. An estimate is made of the energy tolerance of a quantum transition.

Introduction

Although mathematical calculations dealing with the decay of meta-stable states have been published repeatedly, no treatment has appeared which stated explicitly the assumptions as to mechanism on which the formulæ were based. Hence it has been considered advisable to publish such a treatment with special reference to the phenomena to be expected at low temperatures, a problem which is being experimentally attacked by the writer at the present time.

Assumptions

1. The Kinetic Theory of gases is assumed to hold good. This limits the pressures used to those at which the mean free path of a molecule is small compared to the dimensions of the vessel. An upper limit is also imposed by the saturation vapor pressure of the liquid at the temperature studied since Kinetic Theory formulæ would fail for a saturated vapor.

2. Any meta-stable particle striking the walls of the vessel or other liquid or solid surface is immediately returned to the lowest state, the energy being taken up by the wall or surface struck. This will be true for all types of particles regardless of their quantum states, as solid bodies, due to the very large number of degrees of freedom which characterize them, can take up practically any quantum which may be presented to them.

3. In the treatment of temperature variations it seems necessary to make an assumption different from that used by other investigators (1, 2) as to the collisions among atoms which will be effective in causing transfers from meta-stable states to other states. The assumption made in this paper is that only a small number of collisions will be effective in causing such a quantum change

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Contribution from the Physics Laboratories of the University of Toronto, Toronto, Canada.
[2] *Post-graduate student, University of Toronto, and holder of a scholarship under the National Research Council of Canada.*

in the atom and the characteristic feature of these collisions is that the energy available lies within limits which are close to the critical value necessary for the change.

4. As a result of this last assumption (No. 3) it is seen that the maximum number of effective collisions made by meta-stable atoms when the meta-stable state lies below the selected state to which we are considering transfers is given by the number of collisions, whose available kinetic energy lies within a small limit of tolerance of the electronic energy difference between the meta-stable state and the other state under consideration. This number will be multiplied by a fraction depending on the efficiency of various orientations at collision.

5. In cases in which the meta-stable state lies above the one to which we are considering transfers the number of effective collisions is assumed in this paper to be the same as the number occurring if the energy difference involved remained the same and the levels were reversed. This means that, if the system were in true equilibrium with its radiation, the amount of electronic energy degraded into heat by such a transition would be balanced by the kinetic energy transformed into electronic energy by the same transition reversed.

6. In the examples studied by the writer collisions with atoms will be unable to transfer the meta-stable atom directly to the lowest energy state without some intermediate process involving radiation from a non-meta-stable level. The energy which it would be necessary to degrade into heat to accomplish such a transfer is, in these cases, enormous compared to the mean kinetic energy of collision at room temperatures. The number of collisions which could effect the change is fixed by assumptions 3 and 5, and for such large energies (greater than 10 volts in every case) it becomes almost infinitesimal. If a previous transfer to a non-meta-stable excited state takes place the transition falls under the classes considered in assumptions 4 and 5.

7. In the interchange between meta-stable states the same laws (see assumptions 4 and 5) of exchange hold but these transitions require special treatment because the long life of the states allows a large number of exchanges affecting the life of both.

8. Phenomena in cases in which molecules are present will be very complicated due to the increased probability of resonance effects among the close levels of the vibrational and rotational structure of the molecule, as there is then a much greater chance of a required energy difference being exactly available. An instance of this may be cited in the enormously increased absorption of certain mercury lines in the presence of nitrogen. This is due in part at least to the fact that the difference in energy represented by $^3P_0 - ^3P_2$ in the mercury atom is equal within two frequency units to that possessed by a neutral nitrogen molecule in the second vibrational level of the zero electronic state.

Theory

In the case of the inert gases which are being studied we have in each case two meta-stable states. It is necessary, therefore, to solve the two simultaneous differential equations,

$$\frac{\partial N_1}{\partial t} = K_1\left(\frac{\partial^2 N_1}{\partial x^2} + \frac{\partial^2 N_1}{\partial y^2} + \frac{\partial^2 N_1}{\partial z^2}\right) - C_1N_1 + B_1N_2$$

$$\frac{\partial N_2}{\partial t} = K_2\left(\frac{\partial^2 N_2}{\partial x^2} + \frac{\partial^2 N_2}{\partial y^2} + \frac{\partial^2 N_2}{\partial z^2}\right) - C_2N_2 + B_2N_1$$

Since cylindrical tubes are used whose length is very great compared to the radius we may transfer to cylindrical coordinates and neglect diffusion to the ends. Variation with change of θ will also be absent as seen from symmetry. This gives

$$\frac{\partial N_1}{\partial t} = K_1\left(\frac{\partial^2 N_1}{\partial r^2} + \frac{1}{r}\frac{\partial N_1}{\partial r}\right) - C_1N_1 + B_1N_2$$

$$\frac{\partial N_2}{\partial t} = K_2\left(\frac{\partial^2 N_2}{\partial r^2} + \frac{1}{r}\frac{\partial N_2}{\partial r}\right) - C_2N_2 + B_2N_1$$

in which N_1 is the number of meta-stable atoms in one state, N_2 is the number of meta-stable atoms in the other state, K_1 and K_2 are the diffusion coefficients of the two types of atoms in the unexcited gas. C_1 and C_2 are the number of collisions per second of the meta-stable atom which are effective in removing the atom from that state. B_1 and B_2 are the number of collisions per second per meta-stable atom which are effective in putting the atom in the other meta-stable state. The quantities of K, C, and B can on the basis of the above assumptions be evaluated from the Kinetic Theory. The expressions are:— (3)

$$K = \frac{\sqrt{8kTA/\pi\left(1/M_1 + 1/M_2\right)}}{3\pi N\sigma^2}$$

$$C = \sum_i 2N\sigma^2\left(\frac{\pi m}{kT}\right)^{1/2} e^{-(mR_i^2/4kT)} R_i\delta R_i$$

$$B = 2N\sigma^2\left(\pi m/kT\right)^{1/2} e^{-(mR_o^2/4kT)} R_o\delta R_o$$

in which N is the total number of atoms per cc., m is the mass of an atom, M is the molecular weight of the gas in grams, k is Boltzmann's constant, T is the absolute temperature, σ is the average diameter of a molecule, A is Avogadro's number, R_i is the critical velocity along the line of centres necessary to cause a quantum change.

It is given by $\frac{mR_i^2}{2} = h\nu_i c$ where ν_i is the frequency difference of the levels in cm^{-1}, h is Planck's constant, and c is the velocity of light.

R_o is the particular value of R_i which will cause a transfer between the two meta-stable states, δR is the small range of velocity within which the collision will be effective in producing a quantum transition. $\sum_i$ is summed over all possible transitions.

The equations above are solved as usual, subject to the boundary conditions in a long cylindrical tube of radius a.

N_1 and $N_2 = 0$ at the walls of the vessel i.e., at $r = a$, $N_1 = N_0$, for any point not on the walls of the tube for $t = 0$, $N_2 = N_0$, for any point not on the walls of the tube for $t = 0$.

The assumption that $N_1 = N_0$, a constant at $t = 0$, is doubtless very rough but, as experiments do not deal with actual numbers but with changes with time, the results in this respect should be quite accurate. In the equations we may separate the variables by the usual device of putting $N_1 = T_1R_1$ and $N_2 = T_2R_2$ in which T_1 and T_2 are functions of time only, and R_1 and R_2 are functions of r only. Since r is involved in precisely the same way in the two equations with identical boundary conditions upon it R_1 is taken to be identical with R_2, which simplifies the solution of the equations very much.

One arrives at the relations

$$\frac{1}{K_1T_1}\frac{dT_1}{dt} = \frac{1}{R}\left(\frac{d^2R}{dr^2} + \frac{1}{r}\frac{dR}{dr}\right) - \frac{C_1}{K_1} + \frac{B_1}{K_1}\frac{T_2}{T_1}$$

$$\frac{1}{K_2T_2}\frac{dT_2}{dt} = \frac{1}{R}\left(\frac{d^2R}{dr^2} + \frac{1}{r}\frac{dR}{dr}\right) - \frac{C_2}{K_2} + \frac{B_2}{K_2}\frac{T_1}{T_2}$$

whence one derives

$$\frac{1}{K_1T_1}\frac{dT_1}{dt} + \frac{C_1}{K_1} - \frac{B_1}{K_1}\frac{T_2}{T_1} = -p^2$$

$$\frac{1}{K_2T_2}\frac{dT_2}{dt} + \frac{C_2}{K_2} - \frac{B_2}{K_2}\frac{T_1}{T_2} = -p^2$$

$$\frac{1}{R}\left(\frac{d^2R}{dr^2} + \frac{1}{r}\frac{dR}{dr}\right) = -p^2$$

The solution of the third equation is of course $J_0(pr) = R$. The boundary condition $R = o$, at $r = a$, fixes p as equal to $\frac{\mu_n}{a}$ in which μ_n is one of the infinite sequence of roots of $J_0(x) = 0$. The solution for T_1 is obtained by eliminating T_2 and its derivatives from the two equations and equations obtained by differentiating them.

T_1 is found to be the solution of

$$\frac{d^2T_1}{dt^2} + \left((C_2 + p^2K_2) + (C_1 + p^2K_1)\right)\frac{dT_1}{dt} + \left((C_1 + p^2K_1)\,(C_2 + p^2K_2) - B_1B_2\right)T_1 = 0$$

that is

$$T_1 = e^{\left(\frac{-(C_2 + p^2K_2 + C_1 + p^2K_1) + \sqrt{((C_2 + p^2K_2) - (C_1 + p^2K_1))^2 + 4B_1B_2}}{2}\right)t}$$

For reasons of symmetry it is seen that T_2 will be of the same form with C_1 and C_2, B_1 and B_2, K_1 and K_2 interchanged.

The solution for N_1 becomes

$$\left\{ N_1 = T_1 R = \sum_n A_n J_o\left(\frac{\mu_n}{a} r\right) \cdot \right.$$
$$\left. \cdot e^{\left(\frac{-(C_2+p^2K_2+C_1+p^2K_1) \pm \sqrt{((C_2+p^2K_2)-(C_1+p^2K_1))^2+4B_1B_2}}{2}\right)t} \right.$$

in which A_n is determined (1) from

$$N_o = \sum_n A_n J_o\left(\frac{\mu_n}{a} r\right), \text{ to be } A_n = \frac{2}{\mu_n J_1(\mu_n)}.$$

This expression does not give anything measurable and it is necessary to carry the integration throughout the volume of the light beam passing through the meta-stable atoms in order to get a relation involving the absorption, a factor which is subject to observation. It is assumed that the number of atoms removed from the meta-stable state by the light beam is small compared to the total number in that state. The following expression is arrived at

$$-dI = 2\pi IP\left(\int_o^{a_1} Nrdr\right) dl$$

in which I is the intensity of the light at any section, P a factor giving the probability of absorption, dI the amount of light removed from the beam in a length dl. a_1 is the radius of the incident beam. Carrying out this integration one gets

$$\left\{ -\log \frac{I}{I_o} = \sum_n \frac{4\pi N_o P a a_1}{\mu_n^2} \frac{J_1\left(\mu_n \frac{a_1}{a}\right)}{J_1(\mu_n)} \cdot \right.$$
$$\left. \cdot e^{\left(\frac{-(C_1+C_2+p^2(K_1+K_2)) \pm \sqrt{((C_2-C_1)+p^2(K_2-K_1))^2+4B_1B_2}}{2}\right)t} \right.$$

It is easily shown that for any value of the time which can be experimentally measured this series is so rapidly convergent that all but the first term may be neglected. It may also be pointed out that this function, $-\log \frac{I}{I_o}$ is particularly easy to evaluate from photographic measurements as the formula generally used, connecting intensity and blackening of plates is,

$$\log \frac{I}{I_o} = \frac{D-D_o}{\gamma}$$

in which D is a measure of the darkening and γ a constant. As Zemansky (2) points out, a complication is introduced by evaluating the change with time of the function $\left(1 - \frac{I}{I_o}\right)$ as was done by a previous investigator. It is evident that the half-life period of this function will depend not only on the constants of the tube and contents, but on the relative probability of absorption for each

line so that a dependence of half-life on the line used to observe it will result as was found by Meissner and Graffunder (1). From the above we deduce that the half value time of the above function, $-\log \frac{I}{I_o}$, is

$$\tau = \frac{-2\log_e \frac{1}{2}}{C_1+p^2K_1+C_2+p^2K_2 \pm \sqrt{\left((C_2+p^2K_2)-(C_1+p^2K_1)\right)^2+4B_1B_2}}$$

As a first approximation this becomes

$$\tau = \frac{-\log_e \frac{1}{2}}{C_1+(\mu_1^2/a^2)K_1 \pm \frac{B_1B_2}{(C_2-C_1)+(\mu_1^2/a^2)(K_2-K_1)}}$$

The alternative sign is determined by the sign of the denominator of the final term. The resultant sign of this term must obviously be negative since the effect is in all cases to reduce the number of effective collisions which take place. It is seen from the above assumption (No. 4) that $B_1 = B_2$. It is assumed for the purpose of preliminary calculation that the two diffusion coefficients K_1 and K_2 are equal. The relation then reduces to

$$\tau = \frac{-\log_e \frac{1}{2}}{C_1+(\mu_1^2/a^2)K_1 \pm B^2/(C_2-C_1)}$$

On substituting numerical values for the known constants in the expression given above

$$C \text{ becomes } \frac{2.99\times10^{21}}{\sqrt{TM}} P\sigma^2 \sum_i e^{-\frac{.713\nu_i}{T}\sqrt{\nu_i\delta\nu_i}}$$

$$B \text{ becomes } \frac{2.99\times10^{21}}{\sqrt{TM}} P\sigma^2 e^{-\frac{.713\nu_o}{T}\sqrt{\nu_o\delta\nu_o}}$$

in which P is the gas pressure in mm. of mercury measured at 300° A.

$$K \text{ becomes } \frac{6.72\times10^{-14}}{P\sigma^2}\sqrt{\frac{T}{M}}$$

The above method of treating the decay due to collisions makes it possible to set a lower limit and even evaluate approximately the quantity $\delta\nu$ which might be called the average tolerance of the quantum transition. This factor as it appears in the equations is composed of two parts, a fraction depending on the orientation of the atoms at the collision, and the true frequency tolerance, that is, the average energy in frequency units which the transition allows to be degraded into heat. If all orientations are equally efficient the whole of $\delta\nu$ as it appears in the equation will be due to this frequency tolerance, thus making it possible to set a lower limit which this quantity must exceed.

On inserting the radius of the tube used in experiments to be published later, a = 2.5 cm., and putting $\mu_1 = 2.405$, the above formula becomes

$$\tau = \frac{0.699}{\frac{2.99\times10^{21}P\sigma^2}{\sqrt{1M}}\sum_i e^{-\frac{.713\nu_i}{T}\sqrt{\nu_i\delta\nu_i}} + \frac{6.22\times10^{-14}}{P\sigma^2}\sqrt{\frac{T}{M}} \pm \sqrt{\frac{2.99\times10^{21}P\sigma^2}{\sqrt{TM}}\left(e^{-\frac{.713\nu_o}{T}\sqrt{\nu_o\delta\nu_o}}\right)^2 \Big/ \sum_j e^{-\frac{.713\nu_j}{T}\sqrt{\nu_j\delta\nu_j}} - \sum_i e^{-\frac{.713\nu_i}{T}\sqrt{\nu_i\delta\nu_i}}}}$$

Results

For helium the following transitions are the only ones having an appreciable effect at the temperatures studied.

$2^3S - 2^3P -$ 9,231 cm^{-1}
$2^3S - 2^1P -$ 11,279 cm^{-1}
$2^3S - 2^1S -$ 6,421 cm^{-1}
$2^1S - 2^1P -$ 4,857 cm^{-1}
$2\,S - 2^3P -$ 2,810 cm^{-1}

By the use of these transitions the values of the function $\sum_i$ may be deduced. A special treatment is necessary for the $2^3S - 2^3P$ transition as many of the atoms making this jump will immediately return with the emission of λ10830. The total effect of this transition is small at ordinary and low temperatures.

TABLE I

Function	Value at 300°A.	Value at 80° A.	Value at 21° A.
$2^1S_0 \sum_i e^{-\frac{.713\nu_i}{T}\sqrt{\nu_i}}$	6.78×10^{-2}	7.34×10^{-10}	5.5×10^{-40}
$e^{-\frac{713\nu_0}{T}\sqrt{\nu_o}}$	3.8×10^{-5}	1.27×10^{-23}	2.5×10^{-93}
$2^3S_1 \sum_j e^{-\frac{.713\nu_j}{T}\sqrt{\nu_j}}$	3.8×10^{-5}	1.27×10^{-23}	2.5×10^{-93}

This table shows the very rapid decrease in the effect of collisions as the temperature is lowered. The diameter of the helium atom is taken as 2.15×10^{-8}cm. (Average value, Smithsonian tables).

The value of the factor $\sqrt{\delta\nu}$ is taken to be $\frac{1}{200}$. Allowing a tolerance of approximately 8° of arc in orientation this gives the true $\delta\nu$ as being of the order of ¼ frequency unit or 10^{-5} volts.

The diffusion coefficient as given by the formulæ above has been multiplied by a correction factor $0.82 \pm .02$. This has been deduced from experimental values of various diffusion coefficients listed in tables.

With these numerical values substituted in the equation above, the half-life times for the 2^1S_0 state of helium are given in Table II.

TABLE II

HALF-LIFE TIME VALUES OF HELIUM ATOMS

Pressure	τ at 300° A.	τ at 80° A.	τ at 21° A.
1 mm.	7.22×10^{-4} sec.	14.2×10^{-4} sec.	27.7×10^{-4} sec.
2 mm.	13.9×10^{-4} sec.	28.4×10^{-4} sec.	55.4×10^{-4} sec.
3 mm.	19.5×10^{-4} sec.	42.6×10^{-4} sec.	83.0×10^{-4} sec.
4 mm.	24.2×10^{-4} sec.	56.8×10^{-4} sec.	110×10^{-4} sec.
5 mm.	27.0×10^{-4} sec.	71.0×10^{-4} sec.	138×10^{-4} sec.
6 mm.	29.2×10^{-4} sec.	85.2×10^{-4} sec.	166×10^{-4} sec.
7 mm.	30.2×10^{-4} sec.	99.3×10^{-4} sec.	194×10^{-4} sec.
8 mm.	30.6×10^{-4} sec.	113.0×10^{-4} sec.	222×10^{-4} sec.
9 mm.	30.6×10^{-4} sec.	128.0×10^{-4} sec.	249×10^{-4} sec.
10 mm.	30.3×10^{-4} sec.	142.0×10^{-4} sec.	277×10^{-4} sec.
20 mm.	22.0×10^{-4} sec.	$284. \times 10^{-4}$ sec.	554×10^{-4} sec.

NOTE: $^1S_0$ state of helium.

A similar table is deduced for the $^3S_1$ state of helium.

TABLE III

HALF-LIFE TIME VALUES OF HELIUM ATOMS

Pressure	τ at 300° A.	τ at 80° A.	τ at 21° A.
1 mm.	7.34×10^{-4} sec.	14.2×10^{-4} sec.	27.7×10^{-4} sec.
2 mm.	14.68×10^{-4} sec.	28.4×10^{-4} sec.	55.4×10^{-4} sec.
3 mm.	22.0×10^{-4} sec.	42.6×10^{-4} sec.	83.0×10^{-4} sec.
4 mm.	29.4×10^{-4} sec.	56.8×10^{-4} sec.	110×10^{-4} sec.
5 mm.	36.6×10^{-4} sec.	71.0×10^{-4} sec.	138×10^{-4} sec.
6 mm.	44.0×10^{-4} sec.	85.2×10^{-4} sec.	166×10^{-4} sec.
7 mm.	51.3×10^{-4} sec.	99.3×10^{-4} sec.	194×10^{-4} sec.
8 mm.	58.6×10^{-4} sec.	113.0×10^{-4} sec.	222×10^{-4} sec.
9 mm.	66.0×10^{-4} sec.	128×10^{-4} sec.	249×10^{-4} sec.
10 mm.	73.4×10^{-4} sec.	142×10^{-4} sec.	277×10^{-4} sec.
20 mm.	146.8×10^{-4} sec.	284×10^{-4} sec.	554×10^{-4} sec.

NOTE: $^3S_1$ *state of helium.*

For neon the frequency differences are

$^3P_2 - ^3P_1$, 417.45 cm^{-1}
$^3P_2 - ^3P_0$, 776.80 cm^{-1}
$^3P_2 - ^1P_1$, 1,846.88 cm^{-1}
$^3P_0 - ^3P_1$, 359.35 cm^{-1}
$^3P_0 - ^1P_1$, 1,070.07 cm^{-1}

From these are deduced the values for $\sum_i$, given in Table IV.

TABLE IV

Function	Value at 300° A.	Value at 80° A.	Value at 21°A.
$^3P_2 \sum_i e^{-\frac{.713\nu^i}{T}} \sqrt{\nu_i}$	12.66	.516	1.42×10^{-6}
$e^{-\frac{.713\nu_o}{T}} \sqrt{\nu_o}$	4.40	.027	6×10^{-11}
$^3P_0 \sum_j e^{-\frac{.713\nu_j}{T}} \sqrt{\nu_j}$	15.08	.805	9.9×10^{-5}

The diameter of the neon atom is taken as 2.15×10^{-8} cm. a value based on the only available determination of the viscosity of neon. The value of $\sqrt{\delta\nu}$ is taken as $\frac{1}{200}$ as before, and the atomic weight of neon is taken to be 20.2. On inserting the above values the half-life times for the $^3P_2$ state are found to be as given in Table V.

TABLE V

HALF-LIFE TIME VALUES OF NEON ATOMS

Pressure	τ at 300° A.	τ at 80° A.	τ at 21° A.
.5 mm.	6.6×10^{-4} sec.	14.9×10^{-4} sec.	31.2×10^{-4} sec.
1 mm.	8.4×10^{-4} sec.	22.8×10^{-4} sec.	62.4×10^{-4} sec.
2 mm.	6.7×10^{-4} sec.	24.4×10^{-4} sec.	124.8×10^{-4} sec.
3 mm.	5.1×10^{-4} sec.	20.8×10^{-4} sec.	187×10^{-4} sec.
4 mm.	4.0×10^{-4} sec.	17.2×10^{-4} sec.	250×10^{-4} sec.
5 mm.	3.3×10^{-4} sec.	14.4×10^{-4} sec.	312×10^{-4} sec.
6 mm.	2.8×10^{-4} sec.	12.3×10^{-4} sec.	374×10^{-4} sec.
7 mm.	2.4×10^{-4} sec.	10.7×10^{-4} sec.	436×10^{-4} sec.
8 mm.	2.1×10^{-4} sec.	9.5×10^{-4} sec.	497×10^{-4} sec.
9 mm.	1.9×10^{-4} sec.	8.5×10^{-4} sec.	561×10^{-4} sec.
10 mm.	1.7×10^{-4} sec.	7.7×10^{-4} sec.	624×10^{-4} sec.

NOTE: *state of neon atom*, $^3P_2$.

For the $^3P_0$ state of neon similar calculations give the values listed in Table VI.

TABLE VI

HALF-LIFE TIME VALUES OF NEON ATOMS

Pressure	τ at 300° A.	τ at 80° A.	τ at 21° A.
.5 mm.	5.9×10^{-4} sec.	13.7×10^{-4} sec.	31.2×10^{-4} sec.
1 mm.	6.6×10^{-4} sec.	19.4×10^{-4} sec.	62.4×10^{-4} sec.
2 mm.	4.7×10^{-4} sec.	18.1×10^{-4} sec.	124.8×10^{-4} sec.
3 mm.	3.4×10^{-4} sec.	14.4×10^{-4} sec.	187×10^{-4} sec.
4 mm.	2.7×10^{-4} sec.	11.5×10^{-4} sec.	250×10^{-4} sec.
5 mm.	2.2×10^{-4} sec.	9.5×10^{-4} sec.	312×10^{-4} sec.
6 mm.	1.8×10^{-4} sec.	8.1×10^{-4} sec.	374×10^{-4} sec.
7 mm.	1.6×10^{-4} sec.	7.0×10^{-4} sec.	436×10^{-4} sec.
8 mm.	1.4×10^{-4} sec.	6.2×10^{-4} sec.	497×10^{-4} sec.
9 mm.	1.2×10^{-4} sec.	5.5×10^{-4} sec.	561×10^{-4} sec.
10 mm.	1.1×10^{-4} sec.	5.0×10^{-4} sec.	624×10^{-4} sec.

NOTE: *state of neon atom*, $^3P_0$.

The frequency differences for argon are as follows:

$^3P_2 - {}^3P_1$ 606.8 cm^{-1}
$^3P_2 - {}^3P_0$ 1,409.9 cm^{-1}
$^3P_2 - {}^1P_1$ 2,256.08 cm^{-1}
$^3P_0 - {}^3P_1$ 803.16 cm^{-1}
$^3P_0 - {}^1P_1$ 846.16 cm^{-1}

The atomic diameter of the argon atom is 2.84×10^{-8} cm. (Average value, Smithsonian tables), and the atomic weight, 39.88. Again, taking the value of $\sqrt{\delta_{ii'}}$ to be $\frac{1}{200}$, the values of $\sum_i$ are found to be as given in Table VII.

TABLE VII

Function	Value at 300° A.	Value at 80° A.
$^3P_2 \sum_i e^{-\frac{.713\nu_i}{T}} \sqrt{\nu_i}$	7.16	.108
$e^{-\frac{.713\nu_o}{T}} \sqrt{\nu_o}$	1.34	1.3×10^{-4}
$^3P_0 \sum_j e^{-\frac{.713\nu_j}{T}} \sqrt{\nu_j}$	9.46	.039

By using these results, the half-life values for the $^3P_2$ state of argon are calculated and the values found given in Table VIII.

TABLE VIII

HALF-LIFE TIME VALUES OF ARGON ATOMS

Pressure	τ at 300° A.	τ at 80° A.
.1 mm.	3.9×10^{-4} sec.	7.8×10^{-4} sec.
.2 mm.	6.9×10^{-4} sec.	15.4×10^{-4} sec.
.4 mm.	9.6×10^{-4} sec.	30.0×10^{-4} sec.
.8 mm.	8.9×10^{-4} sec.	54.0×10^{-4} sec.
1.0 mm.	7.9×10^{-4} sec.	61.5×10^{-4} sec.
2.0 mm.	4.6×10^{-4} sec.	72.2×10^{-4} sec.
3 mm.	3.2×10^{-4} sec.	70.5×10^{-4} sec.
4 mm.	2.4×10^{-4} sec.	60.5×10^{-4} sec.
5 mm.	2.0×10^{-4} sec.	52.5×10^{-4} sec.
6 mm.	1.6×10^{-4} sec.	45.7×10^{-4} sec.
7 mm.	1.4×10^{-4} sec.	40.2×10^{-4} sec.
8 mm.	1.3×10^{-4} sec.	35.8×10^{-4} sec.

NOTE: *The $^3P_2$ state of argon.*

For the $^3P_0$ state of argon the results found are given in Table IX:

TABLE IX

HALF-LIFE TIME VALUES OF ARGON ATOMS

Pressure	τ at 300° A.	τ at 80° A.
.1 mm.	3.8×10^{-4} sec.	7.8×10^{-4} sec.
.2 mm.	6.6×10^{-4} sec.	15.5×10^{-4} sec.
.4 mm.	8.5×10^{-4} sec.	30.8×10^{-4} sec.
.8 mm.	7.1×10^{-4} sec.	58.8×10^{-4} sec.
1.0 mm.	6.2×10^{-4} sec.	71.5×10^{-4} sec.
2.0 mm.	3.5×10^{-4} sec.	116×10^{-4} sec.
3 mm.	2.4×10^{-4} sec.	127×10^{-4} sec.
4 mm.	1.8×10^{-4} sec.	123×10^{-4} sec.
5 mm.	1.5×10^{-4} sec.	115×10^{-4} sec.
6 mm.	1.2×10^{-4} sec.	110×10^{-4} sec.
7 mm.	1.0×10^{-4} sec.	99×10^{-4} sec.
8 mm.	$.9 \times 10^{-4}$ sec.	90×10^{-4} sec.

The above tables may be summarized as to their qualitative predictions.

Helium

Diffusion only will be effective in the case of the $^3S_1$ state at the temperatures considered. Collisions will appreciably affect the life of the $^1S_0$ state only at room temperature. At lower temperatures the life of the $^3S_1$ and $^1S_0$ states should be the same. At room temperature the life of the $^3S_1$ state should be longer than that of the $^1S_0$ state.

Neon

Both diffusion and collision have an appreciable effect down to liquid air temperatures for both states. The $^3P_2$ state will have in general a longer life than the $^3P_0$ state. The two half-life times should tend to become the same at the temperature of liquid hydrogen.

Argon

Diffusion and collision will both be effective at all temperatures which can be studied. The maximum half-life value will occur at about the same pressure for the two states at 300° A., but at liquid air temperature the maximum half-life will occur at a slightly higher pressure for the $^3P_0$ than for the $^3P_2$ state.

Criticism of Theory

These calculations assume that the excited atoms conform to a Maxwell's distribution law corresponding to the general temperature of the gas. This is very probably incorrect, as the short periods of excitation used experimentally result in rather high instantaneous voltages. It is evident that if any appreciable fraction of the meta-stable atoms have been ionized before falling into the meta-stable state they will have an added velocity due to the action of the electric field and hence will behave as if at a higher temperature, i.e., diffusion will be faster and the number of collisions whose energy is sufficient to cause a quantum change will be greater. This is probably the explanation of Zemansky's results (2) rather than, as he states, an anomalous value for the diameter of an excited atom. The two effects cannot be differentiated by measurements all made at the same temperature but might be separable in measurements made at a series of temperatures. In this connection it may be mentioned that the half-life time of the $^3S_1$ state of helium is found to be uniformly shorter than that of the $^1S_0$ state, in contradiction to the results given in the above tables. This would indicate that a much larger proportion of the atoms in the $^3S_1$ state had passed through an ionized stage. This is in accordance with the known fact that the intercombination lines between the singlet and triplet systems in helium are very weak. The case of helium is probably the most extreme which will be met since, in order to get a measurable absorption of visible lines, it is necessary to use rather high excitation.

The value of $\sqrt{\delta_{ir}}$ has been taken the same for all transitions and all gases. This is not necessarily true but the fact that this value brings the maximum half-life at approximately the correct pressures in all three cases would indicate that this assumption is at least reasonably sound. In consideration of the above criticism it is seen that $\sqrt{\delta_{ir}}$ is probably smaller than the value selected, but from the calculated results it seems unlikely that this ratio is as great as tenfold. This would set as a lower limit for the average toleration in a collision of this type, a value of 2×10^{-7} frequency units, with a much more probable value in the range of frequency units from 2×10^{-3} to 2×10^{-1}, that is, from 2×10^{-7} volts to about 2×10^{-5} volts.

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THE THEORY OF THE ZONE PLATE DERIVED FROM VOIGT'S INTEGRAL[1]

By C. T. Lane[2]

Abstract

The properties of the zone plate are worked out theoretically, using the method developed by Voigt for diffraction problems. The case treated is the one for which the plate is illuminated by monochromatic plane waves at normal incidence. The various approximations made are clearly indicated and the results obtained are compared with experimental data.

The zone plate, while at present of little importance as a practical optical instrument, is nevertheless interesting in so far that it illustrates that property of wave propagation known as diffraction. The device is accordingly treated in most elementary text books on Optics and very often is included in a laboratory course on that subject. While reviewing a number of the more commonly used text books, the writer was struck with the inadequate character of the treatment, the results obtained by which are often incomplete and in one well-known text (2) incorrect. Indeed there appear to be very few text books in English wherein diffraction theory is treated in a satisfactory manner. Possibly two of the best in this respect are Drude's "Theory of Optics" and Leigh Page's "Introduction to Theoretical Physics"; the treatment in the last named book being excellent and well suited to the student specializing in Physics who is approaching the subject for the first time. In his book Page makes use of an integral, developed some years ago by Voigt, which forms a very satisfactory basis for the treatment of elementary problems in diffraction theory. This integral is derived from the equation of wave propagation $\nabla^2\psi = \frac{1}{V^2}\frac{\partial^2\psi}{\partial t^2}$ without any special assumptions other than ψ shall be an analytic function of its space-time co-ordinates. The integral is also developed in Drude. Since neither of the above mentioned authors applies this integral to the solution of the zone plate and since further such application involves only an elementary type of analysis, the present paper may have some value from the point of view of the teacher of Optics.

Considering a zone plate made up of a series of alternately transparent and opaque zones the radii of whose circles are given by $\rho = \sqrt{p n \lambda}$ wherein p is an arbitrarily chosen number, λ is the wave-length of radiation considered and $n = 1, 2, 3 \ldots$ Those zones the radii of whose smaller circles are given by $n = 2, 4, 6 \ldots$ are opaque, the remainder transparent. Considering also a monochromatic plane wave incident normally on the plate and travelling from right to left, viz.,

$$x = Ae^{2\pi i(\nu t + r/\lambda)}$$

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and Voigt's integral in the form (4)

$$\phi_o = -\frac{iA}{2\lambda}\int_S \frac{1+\cos\,\hat{nr}}{r}\, e^{2\pi i\,(vt+r/\lambda)}\, dS$$

wherein real parts only of the complex numbers are to be considered. From Fig. 1 the following steps will be clear

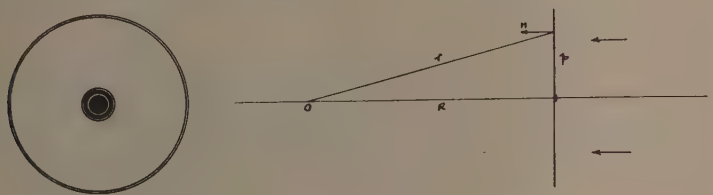

FIG. 1 *Diagram illustrating the plate*

$$\frac{1+\cos\,\hat{nr}}{r} = \frac{R+r}{r^2}$$

$$r = \sqrt{R^2+p^2}$$

$$dS = 2\pi\, p\, dp$$

and ϕ_o is the value of the wave function at O, the integration to be extended over every transparent zone in the plate. It will be sufficient, then, to evaluate ϕ_o' for a single zone (annulus) and then perform a summation over all transparent zones. Accordingly ϕ_o' is evaluated over an annulus of radii c and a respectively, giving rise to the equation

$$\phi_o' = -i\frac{\pi A R e^{2\pi ivt}}{\lambda}\int_a^c \frac{1}{\sqrt{R^2+p^2}}\, e^{\frac{2\pi i}{\lambda}\sqrt{R^2+p^2}}\, d(R^2+p^2)^{1/2}$$

$$-\frac{i\pi A e^{2\pi ivt}}{\lambda}\int_a^c e^{\frac{2\pi i}{\lambda}\sqrt{R^2+p^2}}\, d\sqrt{R^2+p^2}$$

For convenience writing

$$x = \sqrt{R^2+p^2}$$

then,

$$\phi_o' = -i\frac{\pi A e^{2\pi ivt}}{\lambda}\left[R\int_{x_o}^{x_1}\frac{1}{x}\, e^{\frac{2\pi i}{\lambda}x}\, dx + \int_{x_o}^{x_1} e^{\frac{2\pi i}{\lambda}x}\, dx\right]$$

The solution of the second integral in the square brackets can be obtained at once as

$$\int_{x_o}^{x_1} e^{\frac{2\pi i}{\lambda}x}\, dx = \left[\frac{\lambda}{2\pi i}\, e^{\frac{2\pi i}{\lambda}x}\right]_{x_o}^{x_1}$$

The first integral, however, cannot be formed in finite terms and it must therefore be solved approximately as follows:—

Denoting it by J it becomes, by repeated integration by parts,

$$J = \left[\left[e^{\frac{2\pi i}{\lambda} x} \sum_{0}^{n} \frac{D^n \left(\frac{1}{x}\right) i^{n-1}}{\left(\frac{2\pi}{\lambda}\right)^{n+1}} \right]_{x_0}^{x_1} + \left(\frac{\lambda i}{2\pi}\right)^{n+1} \int_{x_0}^{x_1} e^{\frac{2\pi i}{\lambda} x} D^n\left(\frac{1}{x}\right) dx \right]$$

wherein $D^n = \frac{d^n}{dx^n}$

Since λ is a very small quantity a close approximation may be reached by ignoring terms involving λ^2, λ^3 etc. As a close approximation, therefore,

$$J = \frac{\lambda}{2\pi i} \left\{ \frac{1}{x_1} e^{\frac{2\pi i}{\lambda} x_1} - \frac{1}{x_0} e^{\frac{2\pi i}{\lambda} x_0} \right\}$$

and further recalling that

$$x_0 = \sqrt{R^2 + a^2}$$

$$x_1 = \sqrt{R^2 + c^2}$$

the value for ψ_0' finally becomes

$$\psi_0' = \frac{A}{2} e^{2\pi i v t} \left[\left(1 + \frac{R}{\sqrt{R^2+a^2}}\right) e^{\frac{2\pi i}{\lambda}\sqrt{R^2+a^2}} - \left(1 + \frac{R}{\sqrt{R^2+c^2}}\right) e^{\frac{2\pi i}{\lambda}\sqrt{R^2+c^2}} \right]$$

Considering now the Nth zone, the radii of whose circles are $c^2 = 2nP\lambda$ and $a^2 = (2n-1)P\lambda$ respectively. Since both c and a are small numbers their squares may be neglected in comparison with R^2 in the wave amplitude term and hence, finally, for the Nth zone

$$\psi_0' = Ae^{2\pi i v t} \left\{ e^{\frac{2\pi i}{\lambda}\sqrt{R^2+(2n-1)P\lambda}} - e^{\frac{2\pi i}{\lambda}\sqrt{R^2+2nP\lambda}} \right\}$$

The total effect at O of the plate will be, then,

$$\psi_0 = Ae^{2\pi i v t} \left\{ \sum_{1}^{N} e^{\frac{2\pi i}{\lambda}\sqrt{R^2+(2n-1)P\lambda}} - \sum_{1}^{N} e^{\frac{2\pi i}{\lambda}\sqrt{R^2+2nP\lambda}} \right\}$$

wherein, in accordance with what has been said previously, only the real part of this expression is to be considered.

This expression should contain the properties of the zone plate in so far as they can be determined experimentally. Unfortunately the summations cannot be performed and one is forced to expand the expressions inside the radicals and neglect squares and higher terms in λ. Since these quantities occur in the phase term the validity of this step is rather doubtful, but it is the best approximation which it is possible to make.

One gets then that

$$\phi_0 = Ae^{2\pi i\ (vt+R/\lambda)} \left\{ \sum_1^N e^{i(2n-1)\frac{\pi P}{R}} - \sum_1^N e^{i\,2n\frac{\pi P}{R}} \right\}$$

$$= Ae^{2\pi i(vt+R/\lambda)} \frac{sin\ N\alpha}{sin\ \alpha} \left\{ e^{i\,N\alpha} - e^{i\,(N+1)\,\alpha} \right\}$$

wherein $\alpha = \frac{\pi P}{R}$

Taking the real part of this expression:—

$$\phi_0 = A \frac{sin\ N\alpha}{sin\ \alpha} \left\{ cos\ B\ (cos\ N\alpha - cos\overline{N+1}\alpha) - sin\ B\ (sin\ N\alpha - sin\ \overline{N+1}\alpha) \right\}$$

where $B = 2\pi(vt+R/\lambda)$

This expression may be written

$$\phi_0 = A\ \frac{sin\ N\alpha}{sin\ \alpha}\ Q\ cos\ (B+\epsilon)$$

where $Q\ sin\ \epsilon = sin\ N\alpha - sin\ \overline{N+1}\ \alpha$

$$Q\ cos\ \epsilon = cos\ N\alpha - cos\ \overline{N+1}\ \alpha$$

i.e.,

$$Q^2 = 2(1-cos\ \alpha)$$

Hence finally

$$\phi_0 = A\ \frac{sin\ N\alpha}{sin\ \alpha}\ \sqrt{2(1-cos\alpha)}\ cos\ 2\pi \left\{ vt + R/\lambda + \frac{\epsilon}{2\pi} \right\}$$

wherein

$$\epsilon = tan^{-1}\ \frac{sin\ \overline{N+1}\alpha - sin\ N\alpha}{cos\ \overline{N+1}\alpha - cos\ N\alpha}$$

$$\alpha = \frac{\pi P}{R}$$

and the light intensity at any point is given by

$$I \propto 2\ \frac{sin^2 N\alpha}{sin^2 \alpha}\ (1-cos\ \alpha)$$

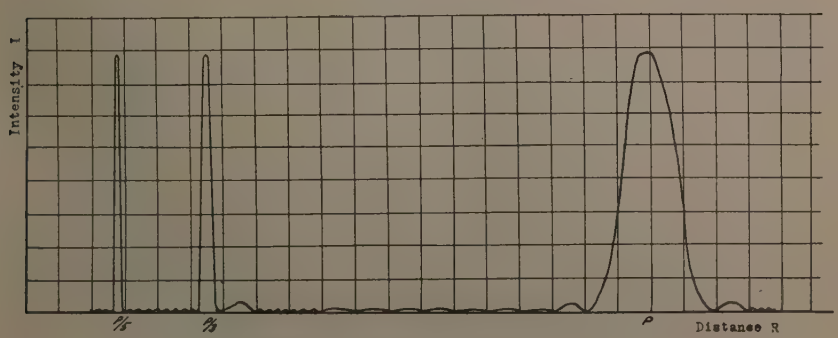

FIG. 2. *Relation of intensity I to distance R from the plate*

In Fig. 2 a curve is plotted showing intensity against distance R from the plate. It is seen that maxima of intensity occur at points along the axis given by $R = \frac{P}{n}$ where $n = 1, 3, 5, \ldots$. This is in accordance with the experimental facts (1, 3). It is seen further that the intensity of each focus should be approximately equal whereas experimentally the intensity decreases as the plate is approached. Fig. 2 is plotted for $N = 10$.

Acknowledgments

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CATALYSTS IN THE PREPARATION OF O-BENZOYLBENZOIC ACID AND ETHYLBENZENES BY THE FRIEDEL AND CRAFTS REACTION[1]

By W. Gallay[2] and G. S. Whitby[3]

Abstract

In the preparation of o-benzoylbenzoic acid from benzene and phthalic anhydride, ferric chloride is a much poorer catalyst than aluminum chloride, but an equimolecular mixture of ferric and aluminum chlorides, although less active than aluminum chloride itself, possesses an activity greater than the sum of the activities of its components separately. Ferric chloride is not a catalyst for the combination of benzene and ethylene. Amalgamated aluminum is a better catalyst than aluminum chloride for both of the reactions mentioned.

The use of ferric chloride as a catalyst in the Friedel and Crafts reaction was first extensively studied by Nencki and his pupils (1, 5*, 8, 9**, 13, 15, 16, 17, 20). Nencki states that, according to his experience, ferric chloride is suitable for the Friedel and Crafts synthesis only with halogen-substituted compounds, and, further, in general gives better results with acid chlorides than with alkyl halides. From benzene and benzoyl chloride a 70% yield of benzophenone was obtained (16). From the same reactants Gangloff and Henderson (6) obtained by means of ferric chloride a 60-62%, and by means of aluminum chloride under similar conditions a 70-71% yield of the ketone. Using ferric chloride to bring about reaction between benzene and carbon tetrachloride, Hinsberg (see Meissel (13)) obtained, after treatment with water, a yield of more than 75% of triphenylcarbinol. Meissel (13) found ferric chloride to act as a catalyst in reaction between benzene and chloroform, the product consisting of a mixture of triphenylmethane and triphenylcarbinol.

Recently Boswell and McLaughlin (3) have described experiments in which a mixture of benzene and chloroform was treated with equivalent amounts of (a) aluminum chloride, (b) ferric chloride, (c) an equimolecular mixture of aluminum and ferric chlorides. They conclude that, although ferric chloride is a much poorer catalyst for the reaction than aluminum chloride, an equimolecular mixture of the two catalysts is as good or somewhat better a catalyst than aluminum chloride alone. These conclusions are based, not on the isolation of the products of the reaction, but on the measurement of the hydrogen chloride liberated. It must, however, be considered doubtful whether such measurements provide a firm basis for comparing the activity of different

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[3]*Director, Division of Industrial Chemistry, National Research Council, Canada.*

Elbs had apparently, prior to Nencki, referred to the use of ferric chloride in Friedel and Crafts reactions, but the reference in question is not available to the present authors.
**Hamonet had previously treated acid chlorides with ferric chloride, but his experiments did not include typical Friedel and Crafts reactions.*

catalysts in the Friedel and Crafts reaction. In experiments on the interaction of benzene and phthalic anhydride under the influence of aluminum chloride, in which different proportions of catalyst and different periods of heating were applied, Rubidge and Qua (21) did not find a strict proportionality between the amount of hydrogen chloride liberated and the weight of the products isolated*.

In the present series of experiments a comparison was made of aluminum chloride, ferric chloride and an equimolecular mixture of aluminum and ferric chlorides in the reaction between benzene and phthalic anhydride, the *o*-benzoylbenzoic acid produced being isolated and weighed. It was found that ferric chloride is much less effective than aluminum chloride, and that the equimolecular mixture, although considerably less effective than aluminum chloride, is more effective than might have been expected from the activity of its components separately—a result in partial accord with Boswell and McLaughlin's conclusion regarding reaction between benzene and chloroform.

A number of experimenters have demonstrated the ability of aluminum chloride to act as a catalyst in various addition reactions of unsaturated compounds (2, 4, 22), but it is not known whether ferric chloride has a similar ability. In order to determine this, the addition of benzene to ethylene was studied. It was found that ferric chloride was devoid of catalytic activity in this reaction. Amalgamated aluminum, however, proved to be an excellent catalyst. It appeared to be more active than aluminum chloride itself, leading to more rapid absorption of ethylene by benzene and the production of more highly ethylated benzenes than did the latter.

The use of amalgamated aluminum, *i.e.*, of a mixture of aluminum metal and mercuric chloride, as a catalyst in the Friedel and Crafts reaction was suggested by Radziewanoski (18), who applied it successfully to the condensation of benzene with ethyl bromide, benzyl chloride and chloroform. Ray (19) has reported the results of further experiments with amalgamated aluminum, mostly on mixtures of benzene with polyhalides such as chloroform and benzal chloride. He found that the products of reaction from such mixtures differed in most cases from the products obtained with aluminum chloride. Thus, for example, both the polyhalides mentioned gave 9:10-diphenyl-9:10-dihydroanthracene, not triphenylmethane (18). Gulewitsch stated that amalgamated aluminum is of no value for condensing aromatic hydrocarbons and acid chlorides, basing the statement on the observation that it gave a negligible yield of acetophenone from benzene and acetyl chloride. In the present experiments amalgamated aluminum is found to be an even better catalyst than aluminum chloride for the union of benzene and phthalic anhydride, the yield of *o*-benzoylbenzoic acid being theoretical.

**Cf., e.g., Rubidge and Qua's experiments No. 6 and 15; No. 4 and 5; No. 4 and 15. Cf. also Heller and Schülke (10), who report that in two experiments, under presumably similar conditions, the hydrogen chloride evolved from a mixture of benzene, phthalic anhydride and aluminum chloride was not identical. Experiments of Hirst and Cohen (11) on the condensation of benzene and benzyl chloride under the influence of an aluminum-mercury couple may also be noticed in this connection. While the hydrogen chloride liberated was 86% of the theoretical, the yield of diphenylmethane was much less, only 36% of pure product being isolated.*

The ability of small proportions of an aluminum-mercury couple to bring about condensation of aromatic hydrocarbons and alkyl chlorides was demonstrated by Hirst and Cohen (12). This material was found in the present experiments to be without catalytic effect on a mixture of benzene and phthalic anhydride.

Experimental

o-Benzoylbenzoic Acid.

Reaction was carried out in a three-necked flask, the mixture of carefully dried benzene (in excess), phthalic anhydride and catalyst being stirred strongly by mechanical means while its temperature was slowly raised to 80° C., a point at which it was kept until evolution of hydrogen chloride had ceased. The reflux condenser was replaced by an ordinary one, and excess of cold water was added through a dropping funnel. The heat evolved was sufficient to remove most of the unchanged benzene. Remaining benzene was removed by steam distillation, and the residue was boiled for four hours, sufficient sodium hydroxide being added to make it slightly alkaline. After filtration and acidification, the product was isolated. The yields obtained with different catalysts are given in the following Table.

TABLE I

EFFECT OF CATALYSTS ON THE FORMATION OF O-BENZOYLBENZOIC ACID

Experiment No.	Catalyst per mol. phthalic anhydride	Yield of o benzoylbenzoic acid in %
1	1 mol Al_2Cl_6	91
2	0.5 mol Al_2Cl_6	30
3	1 mol Fe_2Cl_6	12
4	0.5 mol Al_2Cl_6+0.5 mol Fe_2Cl_6	64
4a	0.5 mol Al_2Cl_6+0.5 mol Fe_2Cl_6	68
5	2 atoms Al (powder)+2 mols $HgCl_2$	100
6	1 atom Al (powder)+ 2 mols $HgCl_2$	10
7	Trace Al-Hg couple	0
8	0.5 mol Al_2Cl_6+1 mol $SnCl_4$	Trace

The yield obtained in Experiment No. 1 agrees with that obtained by Heller (10) and by Rubidge and Qua (21) for similar conditions, while the yield obtained in Experiment No. 2 agrees substantially with that obtained by the latter authors in an experiment in which the same proportion of aluminum chloride was used. The presence of stannic chloride almost completely nullifies the catalytic activity of aluminum chloride (Expt. No. 8).

Ethylated Benzenes

The general procedure followed was that used by Milligan and Reid (14), but the rate of stirring was 600 r.p.m. instead of, as in their experiments, 12,000 r.p.m. Ethylene, dried and freed from sulphur dioxide, was passed, by means of a Witt stirrer, into a mixture of 200 gm dry benzene and the finely-ground catalyst for an arbitrary, fixed period, viz., eight hours, the

temperature being maintained at 75-85° C. The reaction product was then worked up in the way described by Milligan and Reid. The results obtained are given in Table II:—

TABLE II

FORMATION OF ETHYLATED BENZENES

Experiment No.	Catalyst	Ethylene absorbed, in gm	Yield of ethylated benzenes in gm		
			Mono-	Di-	Higher
1	50 gm Al_2Cl_6	40	75	35	8
2	50 gm Fe_2Cl_6	Trace	Trace	0	0
3	50 gm Equimolecular mixt. $Al_2Cl_6+Fe_2Cl_6$	5	14	3	0
4*	50 gm Equimolecular mixt. $Al_2Cl_6+Fe_2Cl_6$	5	14	3	0
5	2 gm Al+35 gm $HgCl_2$	50	56	26	50

**In Experiment No. 4 passage of ethylene was continued for a longer period than 8 hours.*

The rate of absorption of ethylene was noticeably higher in Experiment No. 5 than in No. 1. It appeared from a further experiment that an even smaller proportion of amalgamated aluminum than that used in Experiment No. 5 is capable of giving good results. Ethylene passed for a shorter period than in the tabulated experiments into a mixture of 200 gm benzene, 1 gm aluminum and 20 gm mercuric chloride was absorbed to the amount of 33 gm, the product consisting of 56 gm ethylbenzene, 20 gm diethylbenzenes and 15 gm more highly ethylated benzenes.

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STUDIES ON REACTIONS RELATING TO CARBOHYDRATES AND POLYSACCHARIDES. PART XXVI. STRUCTURE OF ACETONE GLYCEROL[1]

By Harold Hibbert[2] and J. G. Morazain[3]

Abstract

The condensation of acetone with glycerol has been carried out in a neutral medium, at a low temperature, and the product shown to consist entirely of the five-membered isopropylidene-glycerol ketal. Based on this fact, the use of the substance for the determination of the structure of fats is therefore warranted. Isopropylidene-glycerol and its acetate readily hydrolyse on heating with water.

No satisfactory explanation can, as yet, be offered for the non-formation of the six-membered ketal under the conditions outlined.

Acetone, as previously shown by other workers, is capable of forming a six-membered cyclic ketal with glycerol-β-methyl ether, dihydroxy acetone, pentaerythritol and certain carbohydrates.

Introduction

Acetone condensation products (cyclic ketals) have been frequently employed during the last 15 years in order to determine the configuration of fats (11) and carbohydrates (8, 9, 14, 15a, 20, 21, 22). The fact that many of these derivatives are well-marked crystalline substances, certain of which are capable of distillation under low pressure, gave considerable impetus to the application of such derivatives.

Fischer (7, 8) was the first to indicate the importance of these isopropylidene derivatives, his views being predicated on the assumption that when acetone condenses with a polyhydroxy derivative, the resulting product is a five-membered cyclic ketal; in other words, acetone is apparently incapable of forming higher-membered ring ketals. This belief was based in part on the fact that Fischer, Bergmann and Bärwind (10) were unable to bring about a ketal condensation between trimethylene glycol and acetone, in part also on the tacit assumption that five-membered rings are almost invariably formed with much greater ease than the six. It is of interest that this latter argument is essentially the one which led Fischer to assign a furanose ring structure to the methyl and other glucosides (12), an opinion shown by the recent brilliant work of Haworth and co-workers (6, 15b) to be quite erroneous.

The views of Fischer on acetone condensation products were seriously called into question when Böeseken and Hermans (2) in repeating Fischer, Bergmann and Bärwind's work on the condensation of trimethylene glycol and acetone, were successful in synthesising the six-membered ketal. Previously, Böeseken and co-workers (1) had been able to show that acetone tends to combine only

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with those hydroxyl groups occupying the *cis* position and attached to adjacent carbon atoms. As a result of these two lines of investigation the cyclic ketals have been largely used in synthetic work as "type substances" for determining the configuration of fats and carbohydrates.

The investigations on cyclic acetal formation carried out by Hibbert and co-workers (17) during the last ten years have indicated the ease with which simultaneous formation of both five- and six-membered cyclic acetals takes place in the condensation of an aldehyde with a polyhydroxy derivative. Further, the marked tendency of ring migration to occur with either of these isomeric acetals under the influence of traces of acid, resulting in the formation of an equilibrium mixture of the five- and six-membered isomers, has been shown in a number of cases (17). Considerable care is thus necessary in drawing conclusions as to structure based on the properties of carbonyl-polyhydroxy condensation products. While all of the evidence hitherto available pointed to the fact that isopropylidene glycerol, obtained by the condensation of glycerol and acetone in the presence of a small amount of hydrogen ion, is a single, homogeneous product, namely a five-membered ring ketal, it seemed highly desirable that the reaction should be thoroughly reinvestigated, and the absence of the six-membered ketal more definitely proven. This is all the more necessary in view of recent investigations indicating that acetone readily combines with di-hydroxy acetone (13), pentaerythritol (3), glycerol-β-methyl ether, b.p. 165/760 mm. (5), and carbohydrates (15a) under the influence of acid catalysts to yield stable six-membered cyclic ketals.

The previous methods employed for the synthesis of acetals and ketals, from aldehydes and ketones respectively and polyhydroxy compounds, embrace (a) *Action of heat and pressure.* This method is difficult to carry out and gives only poor yields. (b) *The use of acids as catalysts.* A wide range of acid concentration has been employed by different workers ranging from 1% dry HCl in the case of the acetone-glycerol condensation, to that of concentrated sulphuric acid used by Böeseken in the synthesis of trichloroethylidene glycerol (4). This method, using an appropriate concentration, gives the best yield in the shortest time. (c) *The use of readily hydrolysable salts* such as zinc chloride, calcium chloride (1), etc. This method requires a longer time and in some cases the use of high pressure and temperature (24). (d) *The employment of neutral, anhydrous salts* such as copper sulphate (13). The reaction takes place slower than in the presence of an acid catalyst but has the advantage of enabling the condensation to be carried out in a neutral medium.

Since isopropylidene-glycerol, as indicated in the experimental part, readily undergoes hydrolysis when heated with water, and especially in presence of acid, it seemed desirable to carry out its synthesis from acetone and glycerol in a neutral medium; in this way hydrolysis of either the five- or six-membered ring, assuming both are formed simultaneously, would be prevented. The condensation was accordingly carried out, using anhydrous glycerol, acetone and copper sulphate, the latter in considerable excess. It was found that

after refluxing this mixture for 48 hr at 60° C. a yield of 30% acetone glycerol was obtained, which was increased to 55% when the heating was continued for 118 hr.

In a further experiment, carried out by agitating pure glycerol and acetone with excess of anhydrous copper sulphate at room temperature for 123 hr, the yield was 60%.

Analysis of Isopropylidene-Glycerol (prepared in neutral medium)

A kilogram of the product was submitted to careful fractionation under reduced pressure. The individual fractions were found to possess, with the exception of the first, the same boiling-point, refractive index and density. Fraction I was found to contain a slight amount of water. On removal of this with a small amount of anhydrous sodium sulphate, the filtered product was found to possess the same physical properties shown by those of the remaining fractions. The residual oil left at the end of the fractionation (approximately 1% of the original product) was shown by a measurement of the refractive index to be glycerol. *The physical properties (boiling-point, refractive index and density) of the different fractions thus definitely indicate the homogeneous character of the entire product.*

Chemical Proof of the Absence of the Six-Membered Ketal

About 500 gm of the above product (mixture of all of the fractions) was methylated in batches of 100 gm. using dimethyl sulphate and aqueous sodium hydroxide and taking great precautions to keep the medium slightly alkaline throughout the reaction.

The isopropylidene-glycerol methyl ether isolated from each reaction product was found to have identical physical properties and on hydrolysis, yielded in addition to acetone only one product, namely, glycerol-α-methyl ether. The boiling point of the latter (110° C./13 mm.) was quite sharp and its physical properties indicated that it was a pure homogeneous substance, free from the corresponding β-methyl ether which boils at 123° C./13 mm.

As a final proof of the absence of the six-membered isomer in the reaction product a further condensation of acetone and glycerol was carried out at room temperature since according to van Roon (23) low temperatures favor the formation of six-membered acetals and ketals. 50 gm of iso-propylidene-glycerol prepared in this way was benzoylated by means of benzoyl chloride in pyridine solution and the reaction product found to consist entirely of isopropylidene-glycerol-α-benzoate.

It would thus seem to be definitely established that the condensation of acetone with glycerol in both neutral and acid media gives rise to only the five-membered derivative, none of the six-membered ketal being formed, at least in amount recognizable by the customary technique. An explanation of this behavior cannot, as yet, be given, although it is expected that much light will be thrown on the subject as soon as the synthesis and properties of the six-membered isopropylidene ketal have been effected. An investigation is being carried out with this object in view.

Experimental Details

Preliminary experiments indicated that boric acid and anhydrous sodium sulphate cannot be used as condensing agents for the synthesis of isopropylidene glycerol. On the other hand, anhydrous copper sulphate proved to be very satisfactory for this purpose.

Method

184 gm (1 mol) of anhydrous glycerol, and 580 gm. (5 mols) of pure dry acetone were refluxed at 56° C. for 17 hr in presence of 108 gm anhydrous copper sulphate and the product then allowed to stand for 10-12 hr at room temperature. Careful fractionation of the reaction mixture gave 346 gm (38.4%) of isopropylidene-glycerol b.p. 82.5-83°C./13 mm.; 94-96° C./21 mm. 1,024 gm of the crude reaction product, was submitted to a careful fractionation under reduced pressure and ten fractions collected, the physical properties of which were then determined. The results are shown in the following table:

FRACTIONAL DISTILLATION OF ISOPROPYLIDENE-GLYCEROL

Fraction No.	Weight in gm	B.P. Range	$n_D^{27°}$	$d_4^{27.5°}$
0	13	below 92°/25 mm.	1.4292	1.0608
1	96	91.5-92°/22 mm.	1.4311	1.0608
			(1.4324)	(1.0648)*
2	99	92°/22 mm.	1.4320	1.0647
3	116	92-92.5°/22 mm.	1.4320	1.0650
4	101	92.5°/22 mm.	1.4322	1.0656
5	97	93°/23 mm.	1.4328	1.0658
6	94	93°/22 mm	1.4325	1.0655
7	105	93-93.5°/22.5 mm.	1.4324	1.0658
8	103	93.5-94°/22.5 mm.	1.4328	1.0655
9	101	94°/22 mm.**	1.4322	1.0658
10	77	94-96°/22**	1.4324	1.0655
Residue: (10 gm)		light brown color	1.4675	
Losses: 12 gm				

**Numbers in parentheses were obtained after drying with anhydrous Na_2SO_4.*
***The slightly higher b.p. was due to unavoidable superheating.*

The close agreement in boiling-point, refractive index and density of the various fractions serves to indicate the homogeneous character of the product, especially in view of the marked difference in these constants in the case of other isomeric glycerol five- and six-membered cyclic acetals (18, 19). In analogy with other five- and six-membered glycerol acetals, it is to be expected that a six-membered isopropylidene-glycerol ketal, if formed, would have a higher boiling-point than the five-membered isomer, and that if present would presumably be left in the residual oil. The latter did not amount to more than 1% of the original material, and as shown by the value found for its refractive index, consisted of glycerol.

Methylation of the Isopropylidene-Glycerol

500 gm of isopropylidene-glycerol, prepared as above, was methylated in quantities of 100 gm by Haworth's method (16), namely, the use of dimethyl sulphate and a 30% aqueous sodium hydroxide solution. Due to the ease with which isopropylidene-glycerol is hydrolysed, even in aqueous alkaline solution, it was found that better yields could be obtained by carrying out the methylation at room temperature (20° C.), and taking the greatest care to see that the reaction medium was kept slightly alkaline throughout the entire reaction.

Method

100 gm of isopropylidene-glycerol was placed in a three-neck flask fitted with a stirrer and two dropping-funnels, the latter serving for the introduction of the dimethyl sulphate and alkali respectively, these reagents being used in the ratio of three moles of each to one of the isopropylidene-glycerol.

An amount equivalent to 0.1 of the volume of each of the two reagents was added, drop by drop, during the course of a 45-minute period to the iso-propylidene-glycerol, the reaction being carried out at room temperature (20° C.) and the mixture being thoroughly stirred throughout the addition. Frequent tests were made to ensure the alkalinity of the solution throughout. (Total time of reaction about 7.5 hr.) 10 cc. of 20% aqueous sodium hydroxide was then added and the mixture heated in the water-bath at 90° C. for one hour to decompose any remaining dimethyl sulphate and methyl sodium sulphate. On cooling, two layers separated, the upper of which (a) was removed. The lower, alkaline layer (b) was then nearly neutralised by the addition of 30% sulphuric acid, taking care to keep the solution well stirred during the neutralisation, so that it remained alkaline throughout, and was finally left in a slightly alkaline condition. The sodium sulphate crystals present were then dissolved by addition of the minimum amount of cold water, the solution extracted four times with ether, and the ethereal solution added to the upper layer (a). The combined solutions were washed four times with cold water to remove the glycerol mono- and di-methyl ethers formed from the methylation of the glycerol produced as a result of hydrolysis of the iso-propylidene-glycerol.

The ethereal solution was dried with anhydrous sodium sulphate, filtered, the ether removed by distillation and the residue fractionated under reduced pressure. Any trace of glycerol tri-methyl ether present passed over with the small amount of low-boiling fraction. (Glycerol trimethyl ether boils at 148° C./765 mm.). Yield 50-58% b.p. 57° C./14 mm., 154°C./774 mm. The amount of product boiling outside this temperature range did not amount to more than 5%.

Hydrolysis of isopropylidene-glycerol methyl ether

116.8 gm of the product obtained as above was dissolved in twice its volume of 40% ethyl alcohol, one cc. of concentrated HCl added, and the mixture refluxed for two hours. It was then neutralised by shaking with a small

amount of lead carbonate followed by silver carbonate, the product filtered, and the water and alcohol removed by distillation under reduced pressure. The residual oil on distillation boiled constantly at 110° C./13 mm. $D_{4°}^{17°}$ 1.1198; $N_D^{17°}$ 1.4462. Yield 72.8 gm or 85%. These properties prove that the compound is pure glycerol α-methyl ether, b.p. 110° C./13 mm. $D_{4°}^{17°}$ 1.1197; $N_D^{17°}$ 1.4463; as compared with glycerol β-methyl ether b.p. 123° C./13 mm. $D_{4°}^{17°}$ 1.1306; $N_D^{17°}$ 1.4505.

Effect of time factor on yield of isopropylidene-glycerol

Using anhydrous copper sulphate, and refluxing at 56° C. it was found that an increase in the length of time of stirring from 17 to 118 hr increased the yield from 38% to 55%. This possibly could be still further increased by longer agitation. Fischer's yield, using HCl as catalyst, was 77%.

Effect of temperature

Inasmuch as van Roon's (23) results point to a greater tendency for the formation of six-membered ketals at low temperature, an experiment was carried out in which a mixture of glycerol (92 gm), acetone (290 gm) and anhydrous copper sulphate (54 gm) was stirred vigorously at 20° C. for 123 hr. A 60% yield of isopropylidene-glycerol was obtained, b.p. 81-84° C./13 mm. 50 gm of this product on benzoylation in the usual manner with a solution of benzoyl chloride in excess of pyridine yielded a homogeneous, crystalline product, m.p. 42° C. identical with that of pure isopropylidene-glycerol α-benzoate.

Hydrolysis experiment with isopropylidene-glycerol

It was found that isopropylidene-glycerol very readily undergoes hydrolysis on heating with water. This is presumably the reason for the low yields obtained in the methylation process since it is necessary to heat the alkaline solution for some considerable time in order to destroy the excess dimethyl- and sodium-methyl-sulphates present. 50 gm of isopropylidene-glycerol was refluxed for 1.5 hr with 35 gm of water, the condenser cooling-water being kept at a temperature around 70° C. to allow the acetone formed to escape. The mixture, after cooling, was transferred to a distillation flask, and the excess water removed under reduced pressure. The distillation was stopped when the temperature rose suddenly to 155° C./10 mm. Weight of glycerol recovered 29.2 gm or 85%.

Isopropylidene-glycerol α-acetate was found to behave in a similar manner.

Conclusions

1. The condensation of acetone and glycerol may be readily brought about at room temperature, in presence of anhydrous copper sulphate. The reaction product consists only of the five-membered isopropylidene-glycerol ketal, and is identical with that prepared by the condensation of acetone and glycerol in a slightly acid medium.

2. Since the condensation was carried out at a low temperature and in a neutral medium (that is, under conditions in which maximum stability of the six-membered ketal, if formed, may be assumed), the conclusion may safely be drawn that in reactions involving the use of an acetone-glycerol condensation product for the determination of the structure of fats, the isopropylidene-glycerol will invariably consist of the five-membered ketal.

3. This conclusion, however, is not applicable to the structure of acetone-carbohydrate condensation products, where free rotation of the carbon atoms in the carbohydrate is not possible. In these cases the existence of six-membered ketals has already been definitely proven (15).

4. Isopropylidene-glycerol and isopropylidene-glycerol α-acetate readily undergo complete hydrolysis on boiling with water.

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EQUILIBRIA IN SULPHUR DIOXIDE SOLUTIONS[1]

By W. Boyd Campbell[2] and O. Maass[3]

Abstract

The investigation described deals with the equilibria existing in a water solution of sulphur dioxide. The vapor pressures and conductivities of solutions of sulphur dioxide were measured over the temperature range 23° to 135° C. at various concentrations up to 8%. The densities of the aqueous solutions up to 15% were also measured. A discussion of the various equilibria in the light of these data leads to the conclusion that sulphurous acid is one of considerable strength, its apparent weakness being due to the small amount of H_2SO_3 actually present. With rise in temperature the free sulphur dioxide increases and the hydrogen-ion concentration of the sulphite liquor used in current practice is so low that it seems highly improbable that the cooking reaction is due essentially to this factor. Apart from the bearing this work may have on the theory of sulphite cooking and the theory of the equilibria existing in the SO_2–H_2O system, it fills a considerable gap in the data regarding sulphur dioxide.

Introduction

Progress in the art of cooking wood by the sulphite process for the production of cellulose pulp for papermaking and similar uses has been attained chiefly by empirical experience. Very little advance has been made toward the scientific explanation of the mechanism of the main reaction, that between the solution of sulphur dioxide and the lignin of the wood whereby the latter is dissolved.

Cross and Engelstad (6) make the following remarks on the process:

"The production of wood cellulose (pulp) by the bisulphite process may be regarded as having been established as an industry in 1884; the retrospect of 40 years of extension and development justifies the critical estimate of the industry as still lacking in scientific foundation.

". . . . That the basis of the process and the industry is mainly empirical is shown in the methods employed for factory control of the main operation. These are not based on the cellulose or main product, nor upon the lignone-complex (by-product), nor upon the direct reaction (sulphonation) . . . but upon indirect observations upon the inorganic reagents employed which . . . approximately measure the progress of the reaction in terms of the active agents (SO_2–CaO) disappearing . . . These tests and the inferences from them have been discussed with an accumulation of technical literature, in a sense, out of proportion with a matter of only indirect import."

One of the most extended and valuable studies of the process was carried out by Miller and Swanson (20). Although their method of study consisted, in the main, of empirically varying various known factors, one at a time, they have by these means secured a considerable improvement in the practical

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technique of sulphite cooking. They make the following statements which may be taken as indicating a view which is quite general throughout the industry.

p. 7. "It has long been accepted that the fundamental reactions in the ccoking of sulphite pulp are a hydrolyzing action, and a sulphonation which separate the ligneous material from the fibres and transform it into a substance that is soluble in water."

p. 68. "The concept of the reactions of the sulphite pulping process that has at times seemed to the authors of paramount importance is that it is, from one point of view, essentially an acid hydrolysis of wood."

If this is really the case, if hydrolysis brought about by the acidity of the cooking liquor is the primary reaction, then it follows that a knowledge of the concentration of the ions of sulphurous acid under the conditions of temperature and pressure obtaining in a sulphite digester is a prime essential to the elucidation of the mechanism of the process. Or, if, as will be shown later, the concentration of the hydrogen ion under these conditions is so low that acid hydrolysis is unlikely, it is important that this should be known so that the way may be cleared for the formulation of some other theory more in accord with the data.

In spite of the fact that sulphur dioxide is such a well known compound qualitatively there is surprisingly little data in the literature as to the properties of its aqueous solutions. Sims (23) determined the vapor pressure of such solutions with considerable accuracy over a range of pressures up to 200 cm. and temperatures up to 50° C. Hudson (11) determined its solubility in water at pressures approximately atmospheric and temperatures up to 90° C. Oman (21) also determined the solubility at temperatures up to 90° C. as did also Enckell (8) though these latter investigators used less exact methods. Enckell also made determinations of the solubility of sulphur dioxide in solutions of calcium bisulphate, of sulphuric acid of sodium sulphate and of sodium sulphate plus hydrochloric acid.

Smith and Parkhurst (24) also made vapor pressure measurements over solutions of sulphur dioxide in water and in water plus various amounts of bases. C. Maass (16) measured the vapor pressure and conductivity of solutions of sulphur dioxide over all possible concentrations at temperatures below 27°. C. Lindner (15) made determinations of vapor pressure at 0°, 25° and 50° C. of four solutions ranging in concentration from 0.05% to 3.8%. He also made conductivity measurements of the following solutions:—

6.20% at temperatures	0° C. to 39.9° C.
3.24% at temperatures	12.7° C. to 56.7° C.
0.34% at temperatures	9.5° C. to 69.9° C.
0.05% at temperatures	0° C. to 70.5° C.

Other investigations covering parts of the field included in this paper are those by Ostwald (22) and Barth (3) using conductivity methods, McCrae and Wilson (19) using partition proportions of SO_2 between water and chloroform,

Walden and Centnerszwer (26) using freezing point determinations, Drucker (7) who used the combined results of these four investigations as a basis for calculating the dissociation constant.

Kerp and Baur (14) made conductivity and freezing point measurements. Fulda (9) calculating from the heat of dissociation data of Thomsen (25) and Berthelot (4) gives figures for the dissociation constant at temperatures from 7° C. to 50° C. These papers are all discussed by Lindner (15) at some length.

Aside from the fact that there is considerable disagreement among the values for the dissociation constant as calculated by the various methods mentioned above, these data do not, to any appreciable extent, supply information covering the range of solutions of from 0 to 6% concentration at temperatures at or slightly above 100° C., and it is this range which is of most interest to those concerned with the cooking of pulp by the sulphite process.

In discussing the various equilibria involved in the solution of sulphur dioxide in water, it is both usual and convenient to start with the gaseous phase. The partial pressure of the sulphur dioxide above the solution is in equilibrium with the same kind of molecule in the solution

$$[SO_2]_{soln.} = H[SO_2]_{gas} \tag{1}$$

or, if we write p for the partial pressure of the gaseous SO_2

$$[SO_2]_{soln.} = Hp \tag{2}$$

In turn the SO_2 molecules in solution are in equilibrium with sulphurous acid in solution according to the equation

$$H_2O + SO_2 \leftrightarrows H_2SO_3 \tag{3}$$

and applying the mass law

$$[H_2O][SO_2] = K_1[H_2SO_3] \tag{4}$$

Further the sulphurous acid in solution is in equilibrium with its ions,

$$H^+ + HSO_3^- \leftrightarrows H_2SO_3 \tag{5}$$

and, assuming that the Ostwald dilution law holds in this case

$$[H^+][HSO_3^-] = K_2[H_2SO_3] \tag{6}$$

The ionization may proceed further according to the equation

$$H^+ + HSO_3^- \leftrightarrows 2H^+ + SO_3^{--} \tag{7}$$

but for most cases this ionization may be neglected. Ostwald (22) and Barth (3) showed in their work that H_2SO_3 acts as if monobasic even to high dilutions.

To evaluate the individual constants H, K_1 and K_2 is difficult, and in arriving at a value for the dissociation constant most investigators have contented themselves with the determination of the apparent dissociation constant, obtained by the assumption that all the SO_2 in the solution goes to form H_2SO_3.

That is

$$\begin{aligned}[H^+][H\,SO_3^-] &= K_a\left([H_2SO_3] + [SO_2]\right) \\ &= K_a\left(\frac{[SO_2][H_2O]}{K_1} + [SO_2]\right) \\ &= K_a[SO_2]\left(\frac{[H_2O]}{K_1} + 1\right)\end{aligned} \tag{8}$$

and since $$[H^+][H\,SO_3^-] = K_2[H_2SO_3]$$
$$= K_2\frac{[SO_2][H_2O]}{K_1}$$

the relation between K_1, K_2 and the apparent dissociation constant K_a is

$$K_a = \frac{K_2[H_2O]}{K_1 + [H_2O]} \tag{9}$$

C. Maass (16) arrived at an approximation of the individual constants by taking into account the value of $[H_2O]$ as follows:—

$$C_{H_2O} = [H_2O] + [H_2SO_3] + [HSO_3^-]$$
$$C_{SO_2} = [SO_2] + [H_2SO_3] + [HSO_3^-]$$
$$[H_2O] = C_{H_2O} - C_{SO_2} + [SO_2]$$
$$= C_{H_2O} - C_{SO_2} + Hp$$
$$C_{SO_2} = [SO_2] + \frac{[SO_2][H_2O]}{K_1} + \sqrt{K_a\,[SO_2]\left(\frac{[H_2O]}{K_1} + 1\right)} \tag{10}$$
$$= Hp\left(1 + \frac{[H_2O]}{K_1}\right) + \sqrt{K_a\,Hp\left(1 + \frac{[H_2O]}{K_1}\right)}$$

and substituting (10)

$$C_{SO_2} = Hp\left(1 + \frac{C_{H_2O} - C_{SO_2} + Hp}{K_1}\right) + \sqrt{K_a Hp\left(1 + \frac{C_{H_2O} - C_{SO_2} + Hp}{K_1}\right)} \tag{11}$$

Assuming that the value of K_a obtained from conductivity experiments is used, this expression contains two unknowns H and K_1. Accordingly by taking two values of C_{SO_2} at any one temperature together with the corresponding pressures, it is possible to arrive at the value of H and K_1 and hence K_2, the true ionization constant.

Using this method it was shown that the value of K_1 increased rapidly with temperature. That is to say, the proportion of dissolved SO_2 existing in solution as H_2SO_3 became rapidly less as the temperature rose. Calculations from the experimental data by this method showed that at 10° C. the amount of uncombined SO_2 in the solution amounted to about 20% of the total dissolved, whereas at 22° C. the amount of uncombined SO_2 was about 50% of the total. This was for a solution of a concentration of 5% SO_2 in both cases. The value of K_2, the true dissociation constant was found to be approximately 0.02, and did not change much with temperature.

The values obtained by this method are admittedly only approximate, since the variation in the concentration of H_2O is small. In order to obtain a spread of values for the concentration of H_2O sufficiently large to make the calculation possible, it was necessary to work with large concentrations of SO_2—up to 25%. The determination of K_a, the apparent dissociation constant depends on the use of the value for the limiting conductivity of H^+ and HSO_3^- ions as found from conductivity experiments with dilute acids and sulphite solutions. Whether these values for limiting conductivity can be taken as applying without modification to solutions where the concentration of SO_2 amounts to

20-25% is somewhat doubtful. But in spite of these considerations which, after all, reflect only on the degree of accuracy of the determined values, the fact that even approximations have been obtained is of great value.

In this present work the determination of vapor pressures, densities and conductivities of sulphur dioxide solutions of concentrations up to 8% and temperatures to 110° C. are described and the results discussed in a manner somewhat different to the above. This discussion will be left, however, until the experimental portion of the work has been explained.

Experimental Part

Vapor Pressure of Sulphur Dioxide Solutions

The experimental work connected with the securing of the vapor pressure data may, for description, be divided into two parts: the preparation of the solutions, and the measurement of the pressures at the various temperatures.

The solutions, of necessity, had to contain known concentrations of sulphur dioxide and to be free from all impurities which could possibly affect the results. The presence of air in particular had to be avoided. To meet these requirements a method was devised whereby the solution was made by mixing in absence of air a known amount of pure sulphur dioxide gas with a known amount of pure, air-free water in the pressure bulb itself, after which the connecting tubing was sealed off with the blow torch.

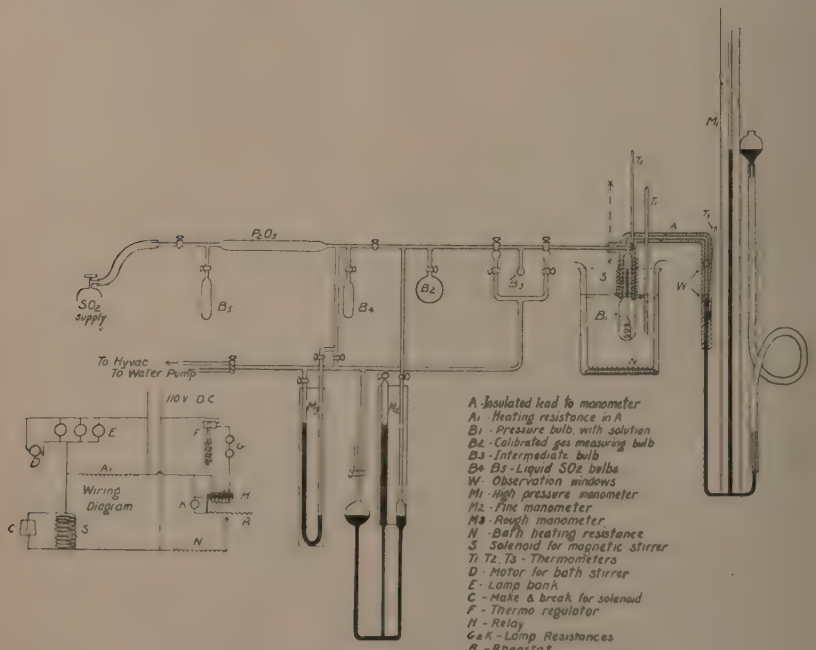
P_2O_5
SO_2 supply
To Hyvac
To Water Pump
110 V D.C.
Wiring Diagram
A - Insulated lead to manometer
A_1 - Heating resistance in A
B_1 - Pressure bulb with solution
B_2 - Calibrated gas measuring bulb
B_3 - Intermediate bulb
B_4 B_5 - Liquid SO_2 bulbs
W - Observation windows
M_1 - High pressure manometer
M_2 - Fine manometer
M_3 - Rough manometer
N - Bath heating resistance
S - Solenoid for magnetic stirrer
T1 T2 T3 - Thermometers
D - Motor for bath stirrer
E - Lamp bank
C - Make & break for solenoid
F - Thermo regulator
H - Relay
G & K - Lamp Resistances
R - Rheostat

Fig. 1. *Diagram illustrating the apparatus used*

The pressure bulb in which the solution was made was connected, by means of an all-glass tube, with an open end mercury manometer capable of measuring the pressures produced. The bulb was immersed in an oil bath of regulated temperature and all tubing from the pressure bulb to the manometer was heated to a temperature above that of the oil bath to ensure the absence of any liquid phase in any portion of the apparatus except the bulb itself. The solution in the bulb was continually stirred by a magnetic stirrer to ensure mixing and true equilibrium. The solution was thus in contact with glass only, and the vapor with glass and the mercury of the manometer.

Apparatus

The details of the apparatus are best described with reference to the diagram, Fig. 1. The bulb B_1 in which the solution was prepared was about one inch in diameter and had a capacity of slightly less than 25 cc. Inside the bulb was placed a magnetic stirrer, the stirrer portion of which was a spiral of glass. This shape was adopted after trying other forms which proved unsatisfactory on account of breaking when the water in the bulb was frozen. The bulb also had a short side arm at an angle of about 45° for the introduction of water. Surrounding the neck of the bulb and extending along the connecting tube to the high pressure manometer was an asbestos jacket in which was imbedded a nichrome resistance wire (No. 30, wound about four turns per inch). This jacket also extended some distance along the manometer. Two windows each about three mm. high were placed in the manometer portion for observation of the mercury column. The manometer tube was protected from local chilling at these windows by the addition of a second larger tube outside, so that the dead air space between acted as an insulator. By means of the imbedded resistance winding the tube could be heated to a temperature sufficiently above that of the solution, so that no condensation would occur, and that the only liquid phase was that in the bulb where the temperature was known.

Thermometers inserted next the tube at two points showed the temperature of the tube. The high pressure manometer was of the ordinary type, except for length. The rubber tubing connecting with the moveable mercury reservoir was the kind used in welding outfits and known as "oxygen hose". It gave no difficulty at the pressures used, and is less expensive than ordinary laboratory tubing.

The bulb was heated in an oil bath consisting of a three-litre pyrex beaker. Heat was applied electrically by means of a coil of about 25 ft. of No. 26 nichrome wire. Temperature control of the bath was effected by a DeKhotinsky thermo-regulator acting through a relay as shown in the wiring diagram. The current used in the heating coil on the tube passed through the solenoid of the magnetic stirrer. Additional current for the stirrer was obtained through a lamp bank. The make-and-break mechanism for the stirrer was made from a metronome so connected as to short-circuit the solenoid at each stroke. This was replaced later by an electric bell, the frequency of

vibration of which was slowed up by a rod attached to the striking hammer and so mounted that the rod swung as a pendulum. This had the advantage of requiring no winding. The solenoid itself consisted of about a pound of No. 18 D.C.C. wire wound about a core in 0.75 in. diameter and 3 in. long, the core being afterwards removed. During determinations of pressure, the upper side connection, through which the SO_2 had been introduced into the bulb, was sealed off, and the tube covered with asbestos and heating resistance wire similar to the remainder of the lead to the manometer.

The portion of the apparatus used for measuring the SO_2 consisted of a tap and rubber tube from the supply cylinder, followed by a glass bulb into which a working quantity of SO_2 could be distilled from the cylinder, after which the tap was closed. Following this bulb was a drying tube of phosphorus pentoxide and another similar bulb, so that the SO_2 could be distilled over the P_2O_5 as often as necessary. A rough manometer and connection to vacuum pumps were attached to this part of the circuit. A tap controlled the connection to the second part of the circuit which was designed particularly for the measurements of the amount of SO_2. This consisted of a bulb of calibrated volume, B_2, with which was connected the fine manometer which could be read to 0.01 cm. Knowing these volumes and the pressure and temperature of the gas, the weight of SO_2 in this part of the apparatus could be readily calculated by means of the data of Maass and Maass (17). At the end of this portion of the apparatus next to the pressure bulb was a small intermediate bulb B_3, on either side of which was a pressure tap. This combination served as a means of getting the gas into the pressure bulb in the manner described below.

Test Procedure

In preparing for a test the procedure was as follows:—

(a) A correction for the effect of heat on one side of the high pressure manometer was obtained by making a reading with the heat on and with atmospheric pressure on each side.

(b) The whole system from the high pressure manometer back was dried out by pumping. A quantity of water was weighed into the pressure bulb through the side opening and then this opening was sealed.

(c) Air was removed from the water by freezing and applying vacuum. This was repeated at least three times in every case. The water bulb with the air-free water was then shut off from the rest of the apparatus by closing the right hand pressure tap.

(d) About 10 cc. of liquid SO_2 was distilled from the supply cylinder into the bulb B_5 after which the cylinder was removed. The whole apparatus was then swept out five times with SO_2 gas from the bulb B_5 thus removing any low boiling impurity from the gas as well as any residual air from the system.

(e) The SO_2 pressure was allowed to rise to the extent desired and the calibrated volume portion of the apparatus closed off. P-V-T readings then gave a measure of the amount of SO_2 in this part of the system.

(f) This gas was then condensed in the small bulb B_3 from which, by closing the tap to the left and opening the one to the right, it was distilled to the pressure bulb and there liquified on top of the frozen water.

(g) The pressure bulb B_1, now containing the requisite amounts of SO_2 and water and cooled to a very low pressure was sealed off at X – X.

(h) The gas remaining in the calibrated volume was then determined by P-V-T readings. The difference between this amount and the previous one being the amount actually in the pressure bulb.

(i) The mixture in the pressure bulb was allowed to melt, the manometer being manipulated to keep the gas volume in due bounds. The insulation and winding of resistance wire were put in place over the sealed off end at X – X.

(k) The oil bath with stirrer, thermometers and thermo-regulator, etc., was now set in position around B_1 and pressure-temperature measurements taken.

Making the test

In making the measurement a large number of points were determined on the low temperature portion of the curve as it was desired to repeat this portion after heating, in order to note any loss in pressure which might be due to decomposition.

It was found in practice that even with no current through the heating coil, N, in the bath, the temperature of the bath rose slowly due to conduction of heat from the hot connecting tube. Accordingly the first part of the curve—to about 32° C.—was determined with the temperature slowly rising in this way. The rise was sufficiently slow for equilibrium to be practically attained at all times.

For higher temperatures the thermo-regulator was set at about the temperature held within about 0.1° C. until a pressure reading was constant for at least two minutes. Readings of temperature and pressure were made in this way at various temperatures about six or seven degrees apart, until the pressure rose to the limit of the manometer. Readings of barometric pressure, room temperature, etc., were also made.

After reaching the maximum pressure, the temperature was lowered to that of the room, the manometer being manipulated to keep the meniscus on the pressure side at approximately the reading point all the time, in the same way as with rising temperature. With the exception of runs No. 9, 10 and 7, no attempt was made to hold it at any point on the lowering temperature for an equilibrium reading. It was found by several trials, however, that reaching a temperature from a higher one gave the same equilibrium as if reached from a lower. This was only to be expected, since the temperature taken was really the mean value of the real temperature which was fluctuating above and below the mean. The fluctuations were so slight, however, that there was no difficulty in maintaining the pressure constant for several minutes. The last point (maximum pressure) for each determination was held for five minutes within a pressure of one mm., ordinarily. In this connexion an interesting

phenomenon is to be noted. The meniscus of the manometer did not remain stationary, but vibrated up and down in synchronism with the movement of the stirrer. This was most evident when the partial pressure of SO_2 was high, as in run No. 11 when the vibration had an amplitude of about three mm. at the higher pressure. This movement was attributed to the greater vapor pressure over a newly formed surface where the orientation of the molecules had not yet taken place. The movement indicated that the pressure was greater when the stirrer was rising.

As mentioned before, a check run was made in each case, in order to note any change in the solution. With the exception of run No. 9 no change was noted, though there was a slight indication of such change in run No. 7. In the case of No. 9, the decomposition was definite, and the check run was carried to a temperature of 127° C. and held there for six hours to note the rate of this decomposition. It was intended to hold this temperature for at least 12 and possibly 24 hours, but the bulb unfortunately exploded.

Calculation

The calculations were fairly simple, though lengthy. The amount of water used needed no calculation as it was weighed directly. The weight of SO_2 was calculated from the formula of Maass and Maass (17).

To get the amounts of SO_2 and water in the solution, it was necessary to deduct from the total obtained as above, the amount of each of these components existing in the gaseous phase. For this purpose the gas volume was divided into two parts—that in the bulb immediately over the solution which was at the same temperature as the solution and that in the heated tube which was at a higher temperature (constant in each test) and which was the average temperature of the tube as shown by the two thermometers in contact with it. For the purpose of calculating the quantities of SO_2 and water in the gaseous phase, it was assumed that the partial pressure of the water was that of pure water at the temperature of the bath, multiplied by the mole fraction of the water in the solution, taking the molecular weight of water as 18 and of SO_2 as 64. The partial pressure of SO_2 was taken as the total pressure minus the partial pressure of water calculated as above. These data, together with the volumes of the respective gas spaces allowed of the calculation of the amount of SO_2 and water in the gaseous phase; by deducting these from the total amounts present, the quantities in the liquid phase were calculated. The volume of the gas phase in the bulb was derived by deducting from the volume of the bulb, the volume of water and of liquid SO_2 added, these being taken at room temperature.

Correction for temperature was made by the usual method for emergent stem correction. Barometric pressure was read on an accurate barometer with a known scale correction. The pressure read on the high pressure manometer was corrected to the equivalent pressure of a column of mercury at 0° C. The scale of the high pressure manometer was of paper and required a factor of 0.996 to correct the readings.

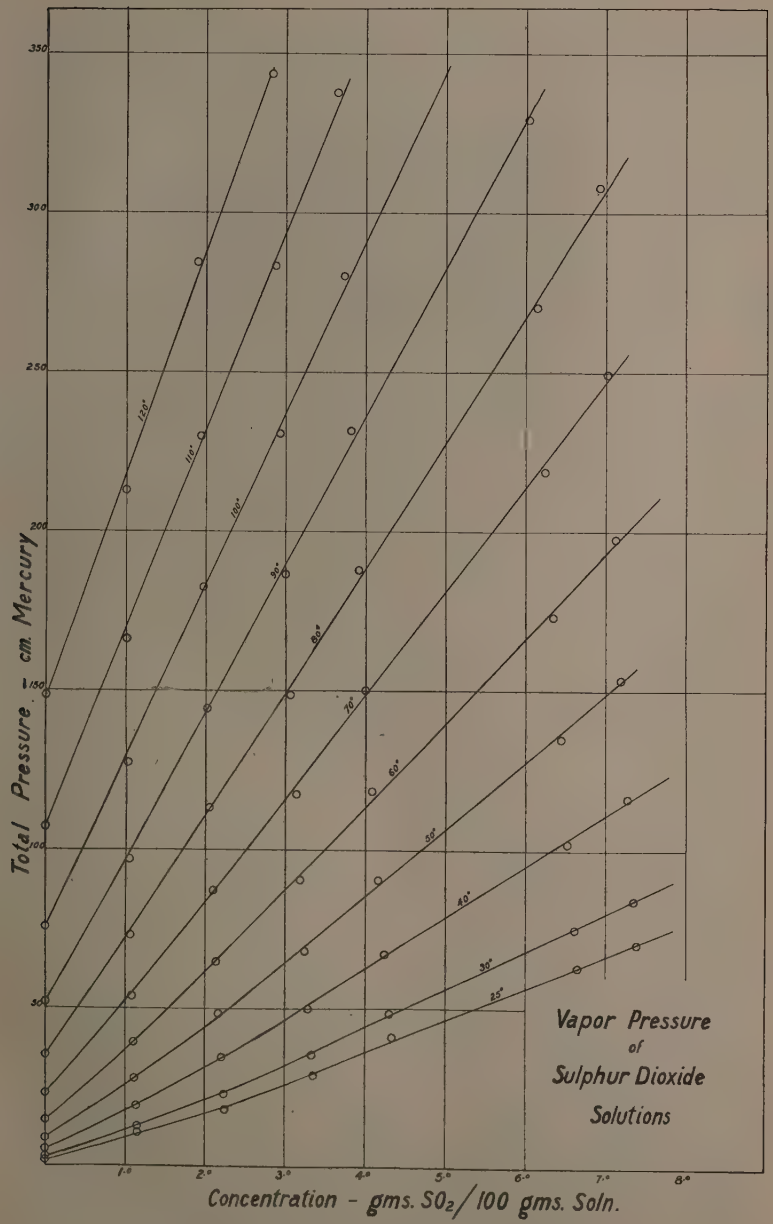

FIG. 2. *Isothermal showing the relation of vapor pressure to composition of aqueous sulphur dioxide solutions.*

The fine manometer scale was graduated in millimeters and etched on a plate glass mirror. It could be read to 0.1 mm. A mercury reservoir, to which suction or pressure could be applied permitted adjustment of the mercury level in the manometer. The mercury on the side connected to the apparatus was always brought to a level in a portion of the tube of the same diameter as the other arm, so as to avoid any necessity for miniscus correction. This same precaution also allowed for the correction for the volume of gas in the manometer.

The method of handling the SO_2 eliminated the only impurity in the supply-water. Tests with $BaCl_2$ showed the absence of SO_3 and even if it had been present it would have remained in the supply bulb since only the middle fraction of the supply was used.

Results

The results were first plotted for each run in two curves, one of which showed the pressure for the particular solution at each temperature, and the other showed the composition of the solution at each temperature. This double lot of curves is made necessary because the composition of the solution changed as the rising temperature forced SO_2 from the solution into the gaseous phase.

From this set of curves a second set were drawn showing pressure corresponding to composition at constant temperature, the isothermals being drawn for each 10° from 30° to 120° with an additional one at 25°. (Fig. 2) To permit reconstruction of these curves the points used are given in Table I.

TABLE I

ISOTHERMAL POINTS OBTAINED FROM EXPERIMENTAL CURVES

Temperature in ° C.		Experiment					
		No. 9	No. 7	No. 6	No. 3	No. 10	No. 11
25	Pressure	10.8	18.4	29.3	41.4	63.5	70.9
	Concentration	1.16	2.25	3.36	4.34	6.66	7.40
30	Pressure	13.0	23.2	35.7	48.7	75.5	84.5
	Concentration	1.15	2.24	3.34	4.31	6.62	7.36
40	Pressure	19.4	34.8	50.0	67.4	102.2	116.5
	Concentration	1.14	2.21	3.29	4.24	6.53	7.28
50	Pressure	28.1	48.7	68.2	90.7	135.0	153.6
	Concentration	1.12	2.17	3.24	4.16	6.45	7.19
60	Pressure	39.6	65.4	90.6	118.6	173.3	197.7
	Concentration	1.11	2.14	3.19	4.08	6.35	7.12
70	Pressure	54.0	87.3	117.6	150.2	218.9	249.4
	Concentration	1.09	2.10	3.14	4.00	6.24	7.02
80	Pressure	73.2	113.2	148.7	188.1	270.3	308.3
	Concentration	1.07	2.06	3.06	3.91	6.14	6.92
90	Pressure	97.1	144.5	186.6	231.8	329.	
	Concentration	1.05	2.02	3.00	3.81	6.03	
100	Pressure	127.5	182.5	230.8	280.1		
	Concentration	1.03	1.97	2.93	3.72		
110	Pressure	166.3	230.0	283.	338.		
	Concentration	1.01	1.93	2.87	3.63		
120	Pressure	213.	284.5	344.			
	Concentration	1.00	1.89	2.82			

Density of Sulphur Dioxide Solutions

The conditions to be met in the determination of the density of sulphur dioxide solutions are that the solutions should be prepared from pure materials; that air or other active gas should be absent; that the volume of definite weights should be accurately measured at elevated temperatures, and that the concentration be measured at the same temperatures.

To meet these conditions the solutions were prepared and the volumes measured in dilatometer bulbs (*e*, Fig. 3) of about one cc. capacity and having capillary tubes about one mm. diameter and 15 cm. long, graduated in mm. divisions. These were calibrated by weighing when filled with mercury.

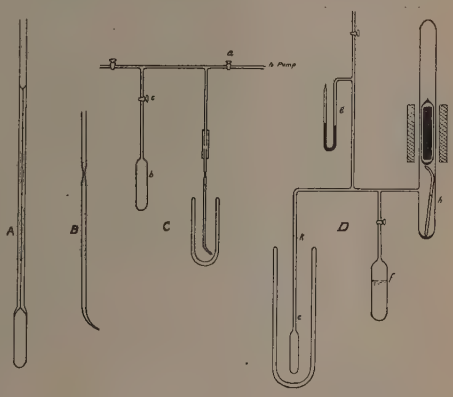

Fig. 3. *Apparatus for Density Determinations*

The dilatometer bulbs were then sealed on to a tube which connected with a bulb of air-free water, *f*, a small manometer, *g*, and a tube in which was a small sealed bulb, *h*, containing a weighed amount of liquid SO_2 and a magnetic hammer for breaking this small bulb. This system was connected with a Hyvac pump. All air having been removed by the pumping and flushing out with water vapor, a quantity of water (judged sufficient) was condensed in the bulb, *e*, by cooling the latter with ice water and opening the connection to the water bulb, *f*. The connection to *f* was then closed and the water in *e* frozen by a CO_2 freezing mixture. The SO_2 bulb *h* was then broken by the magnetic hammer so that the SO_2 distilled over and condensed in *e* on top of the frozen water. Following this the dilatometer *e* was sealed off and disconnected at *k*. Any SO_2 not condensed in the dilatometer was estimated from the pressure as shown on the small manometer, and the known volume of the apparatus.

The dilatometer then contained a known amount of SO_2 and an unknown amount of water. The quantity of water condensed in the bulb was in each case estimated by eye so that the volume of the resulting solution would, as nearly as possible, fill the bulb but not extend into the capillary at room temperature. A smaller quantity than this would have made it necessary to omit volume readings until a fairly high temperature was reached. A greater volume (extending into the capillary) would have made proper mixing uncertain. The dilatometer bulbs thus filled were set aside for three or four weeks, so that the solutions would be thoroughly mixed.

To measure the volumes at the various temperatures the bulbs were immersed in an electrically heated oil bath kept well stirred. The temperature was regulated by a de Khotinsky regulator. Readings were taken at intervals

of about eight or ten degrees, the temperatures being held constant for three minutes for each reading. The only difficulty experienced arose from the occasional liberation of gas bubbles within the body of the liquid. When one of these formed it stopped at the lower end of the capillary and thus broke the liquid column. Except at the higher temperatures this difficulty was overcome by removing the dilatometer from the bath and shaking the liquid into contact again so as to renew the determinations.

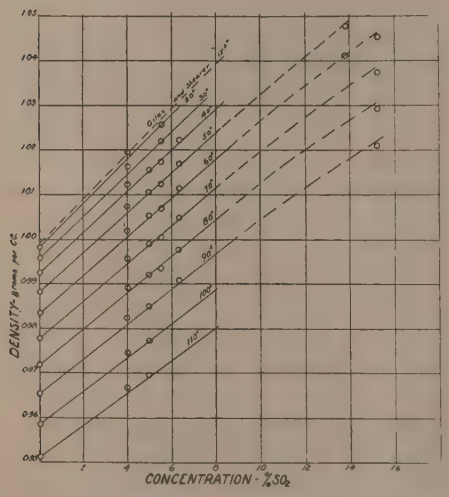

FIG. 4. *Density of sulphurous acid solutions*

After a set of readings had been made in this way the dilatometers were weighed, opened, emptied and dried and then weighed again to obtain the actual weight of solution in each.

The calculations included corrections for the expansion of the glass and for the amount of SO_2 volatilized from the solution. No high accuracy is claimed for this portion of the work but the results were sufficiently accurate for the purpose of the investigation. So far as is known there are no other data on densities at these temperatures.

TABLE II

DENSITY OF SULPHUR DIOXIDE SOLUTIONS

Dilatometer No.	Temperature									
	20° C.		30° C.		40° C.		50° C.		60° C.	
	Density	Concentration in %	Density	Concentration in %	Density	Concentration in %	Density	Concentration in %	Density	Concentration in %
10	1,0197	4.02	1,0163	4.02	1,0123	4.01	1,0073	4.01	1,0019	4.00
5	—	—	—	—	1,0156	4.98	1,0107	4.98	1,0051	4.97
3	1,0258	5.54	1,0218	5.54	1,0172	5.53	1,0122	5.53	1,0068	5.52
7	—	—	—	—	1,0222	6.37	1,0170	6.36	1,0113	6.35
9	—	—	—	—	—	—	1,0459	13.81	1,0411	13.80
8	—	—	—	—	—	—	—	—	1,0453	15.28
	70° C.		80° C.		90° C.		100° C.		110° C.	
10	0,9959	3.99	0,9891	3.99	0,9823	3.99	0,9747	3.99	0,9668	3.99
5	0,9990	4.96	0,9920	4.96	0,9850	4.96	0,9773	4.96	0,9696	4.96
3	1,0004	5.52	0,9933	5.51	—	—	—	—	—	—
7	1,0048	6.35	0,9978	6.34	0,9908	6.33	—	—	—	—
9	—	—	—	—	—	—	—	—	—	—
8	1,0373	15.25	1,0292	15.24	1,0210	15.21	—	—	—	—

The results were plotted for each dilatometer in terms of density and temperature. From these curves a set of isothermals were drawn showing density against concentration at 10° intervals from 20° to 110° C. These curves, plotted from the results given in Table II, are shown in Fig. 4 where the dotted line shows the results of Giles and Shearer at 15.5° C.(10).

Conductivity of Sulphur Dioxide Solutions

The conductivity measurements were carried out in a pyrex cell of the form shown in Fig. 5. In this cell the electrodes are made of very thin platinum foil fused to the inside surface of the cell, a continuation of the same piece of foil passing through the seal to form the lead-in connection. This construction has been previously described by Campbell (5).

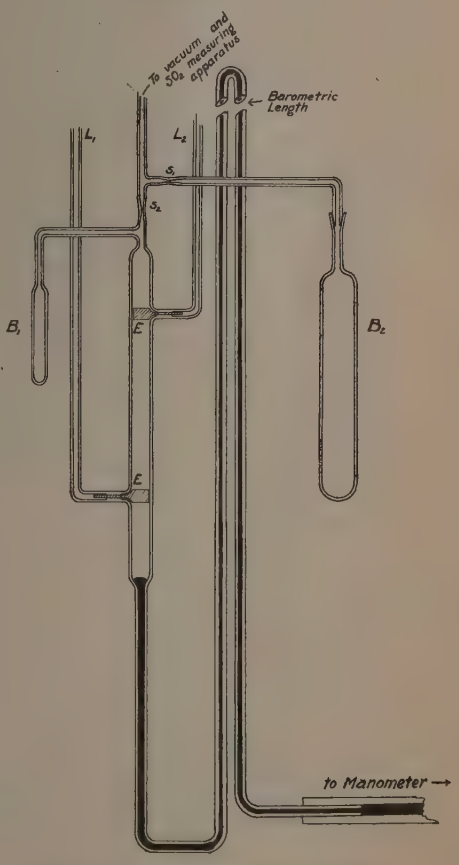

Fig. 5. *Conductivity cell for sulphur dioxide solutions*

The cell was cylindrical with a cross-section of nine mm. diameter and a wall thickness of about 1.1 mm. To allow for the expansion of the solution when heated, and at the same time provide a means for the elimination of gas space, the lower part of the cell was connected with a mercury manometer capable of producing five atmospheres pressure. Interposed between the cell and the manometer was an inverted U-tube of about one mm. bore and a little more than barometric height. There was also a short length of rubber tubing of the kind used for connections on oxy-acetylene welding and known as "oxygen hose". This was capable of standing high pressures but could be closed by pinching with a clamp. This hose was put in the line to provide flexibility and so permit the shifting of the cell from the ice bath to the oil bath as described below. The function of the side bulb B_1 will also become evident in the description of the operation of preparing the solution. The whole apparatus shown, except bulb B_2, was of Pyrex glass.

To prepare a solution the operation was as follows: The cell having been cleaned in the usual manner by washing, steaming, etc., was mounted as shown, and then evacuated completely by a Hyvac pump. At this stage the portion of tubing shown in the figure connected to the mercury pressure column did not contain mercury. This condition was obtained by so regulating the

level in the pressure column that under the influence of the vacuum the mercury did not quite rise to the bend of the inverted U-tube mentioned above. After evacuating (and drying) the apparatus in this way the level of mercury in the pressure column was temporarily raised to a slight extent so as to force a small quantity of mercury over the bend of the inverted U-tube and so down into the bottom of the cell. This quantity of mercury was sufficient to form a seal for the lower end of the cell so that pressure variations up to one atmosphere could take place without affecting the mercury pressure column. Air was then admitted to the cell, bringing it to atmospheric pressure; the bulb B_2 was disconnected at the ground glass joint and an appropriate quantity of recently boiled distilled water weighed into it. The bulb was then replaced as shown in Fig. 5 and well covered with de Khotinsky cement to guard against a possible leak at the ground glass joint. The water in the bulb was then made air-free by repeated freezing and evacuation.

The next step was to distil from the bulb B_2 a sufficient amount of the air-free water into the cell. This was done by surrounding the cell with ice water and immersing the bulb B_2 in a beaker of water slightly above room temperature. Distillation of the necessary water under these conditions usually occupied about a day, the latter part of the operation being very slow on account of the small surface of the cell exposed for the condensation of the vapor. During the distillation the bulb B_1 was kept outside the ice bath so that little or no water condensed in it. After the cell was nearly full of water the bulb B_2 was disconnected by sealing off the tube at the constriction S_1. The residual water in B_2 was weighed, and the amount of water in the cell determined by difference.

A known amount of sulphur dioxide was next added from the calibrated volume apparatus previously described in the section on Vapor Pressure. The entering gas was first condensed in the small bulb B_1 by means of a CO_2-ether freezing mixture and the cell sealed off at the constriction S_2. The rubber hose connected to the pressure column was clamped to prevent the pressure in the cell from forcing the mercury or solution around the lower bend. The liquid SO_2 in the bulb B_1 being allowed to warm slightly, distilled over and dissolved in the water. The cell was then removed from the ice bath and put into an oil bath where the temperature could be adjusted to a fraction of a degree for conductivity measurements. The whole cell from the constriction S_2 down was immersed in the bath, including the bulb B_1 and one leg of the barometric U-tube.

The solution prepared as described was allowed to stand for a couple of days so as to become thoroughly mixed by diffusion. This was perhaps hardly necessary since conductivity readings taken a few hours after the solution had been prepared were practically the same as those taken after several days. After standing the mercury pressure was increased so that mercury entered the lower part of the cell, forcing the solution up to eliminate the gas space and absorb the small amount of gas remaining in it. The cell was allowed to stand another day for further diffusion before measurements of conductivity were made.

A Vreeland oscillator set at a frequency of 1,000 cycles per sec. was the source of current. The ratio arms of the bridge were two special resistances of 100 ohms each and the cell resistance was balanced by a Leeds and Northrup variable resistance box reading to one ohm plus a fine variable resistance (18) by which fractional parts of one ohm could be read. The arrangement was sensitive to 0.01 ohm but readings are given to the nearest 0.1 ohm. A two-stage amplifier was used to make the readings easier. Owing to the decomposition which took place at high temperatures the cell was cleaned and the electrodes replatinized for each solution.

The first run made was that with 4.67% SO_2. The first determinations were made over a range from room temperature up to the maximum. Then the temperature was lowered and the determinations from room temperature down to nearly zero were made. In each case on this lower range the temperature regulation was not quite so sharp as when the bath was being heated but the rate of change of temperature was low and thermometer readings were made almost simultaneously with those of resistance so that no serious error occurred.

On this first run the resistance determined after the various changes of temperature agreed with that originally determined at room temperature. Such was not the case with the second run, that with 2.27% SO_2. This was carried to a higher temperature and there was evident decomposition as shown by a decreasing resistance at constant temperature when near the maximum. On bringing this back to room conditions it was found that the resistance had been appreciably lowered. The lower range was covered as before and the results plotted on the curves. The probable true value of the resistance was calculated from these readings on the assumption that the increased conductivity was due to sulphuric acid and varied at 2% per degree. The conductivity calculated in this way was deducted from the actual readings and is shown as a dotted line on the curve.

Subsequent runs were made by first covering the lower range where no decomposition occurred, so that this difficulty was avoided. Decomposition took place at the higher temperatures in the case of the more dilute solutions but did not take place in the case of the 8.60% solution.

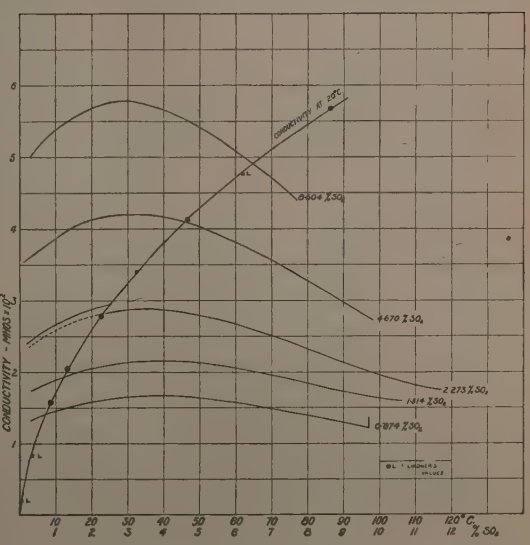

FIG. 6.—*Conductivity of sulphur dioxide solutions*

In the case of the 0.87% solution, it was held at the maximum temperature for 3.5 hr to follow the rate of decomposition.

The purity of the sulphur dioxide used was checked by connecting to the apparatus a bulb containing a solution of barium chloride free from air and saturating it with SO_2 in the same manner as in the preparation of the solutions. Only the faintest opalescence was observed corresponding to an amount of H_2SO_4 far too low to have any effect on the experimental results.

The conductivity results are plotted in Fig. 6, and given in Table III together with calculated values of dissociation constants, etc., which are discussed later.

TABLE III
CONDUCTIVITY AND DISSOCIATION CONSTANTS

Temperature in ° C.	C_{SO_2}	Sp. Cond. $\times 10^2$	$[HSO_3]$	$K_a \times 10^3$	P_{SO_2}(cm.)	$H \times 10^3$
5°	0.1370	1.37	0.0487	26.9	—	—
	.2064	1.79	.0637	28.4	—	—
	.3589	2.44	.0887	27.6	—	—
	.7460	3.69	.1314	28.1	—	—
	1.4023	5.16	.1836	27.6	—	—
	Average	—	—	27.9	—	—
15°	0.1369	1.52	0.0446	21.6	—	—
	.2062	1.98	.0581	22.8	—	—
	.3585	2.70	.0792	22.4	—	—
	.7453	4.02	.1179	22.2	—	—
	1.3996	5.55	.1628	21.4	—	—
	Average	—	—	22.1	—	—
25°	0.1366	1.62	0.0405	17.1	5.6	17.0
	.2058	2.10	.0525	18.0	9.0	17.0
	.3576	2.84	.0710	17.6	17.1	16.8
	.7433	4.18	.1045	17.1	40.9	15.6
	1.3949	5.76	.1440	16.6	80.9	15.5
	Average	—	—	17.3	—	16.4
30°	0.1364	1.65	0.0384	15.1	7.2	13.7
	.2055	2.15	.0500	16.1	11.3	13.7
	.3571	2.87	.0668	15.4	20.9	13.9
	.7420	4.20	.0977	14.8	49.2	13.0
	1.3921	5.76	.1340	14.3	96.4	13.0
	Average	—	—	15.1	—	13.5
50°	0.1353	1.64	0.0302	8.8	14.3	7.3
	.2042	2.13	.0392	9.3	22.4	7.3
	.3542	2.79	.0514	8.7	40.6	7.4
	.7355	4.03	.0742	8.3	90.4	7.3
	1.3790	5.42	.0998	7.8	174.0	7.3
	Average	—	—	8.6	—	7.3
70°	0.1339	1.48	0.0227	4.6	25.1	4.4
	.2017	1.95	.0299	5.2	38.7	4.4
	.3503	2.51	.0385	4.7	67.7	4.6
	.7272	3.57	.0548	4.5	147.3	4.6
	1.3619	4.71	.0722	4.1	276.7	4.6
	Average	—	—	4.6	—	4.5
90°	0.1321	1.29	0.0170	2.5	38.5	3.0
	.1991	1.72	.0227	2.9	58.9	3.0
	.3456	2.12	.0280	2.5	103.8	3.1
	.7170	2.97	.0392	2.3	215.7	3.1
	Average	—	—	2.5	—	3.0

Discussion of Results

The dissociation constant usually determined for sulphurous acid is the apparent dissociation constant.

$$K_a = \frac{[H^+]\,[HSO_3^-]}{[H_2SO_3]+[SO_2]} = \frac{[HSO_3^-]^2}{C_{SO_2} - [HSO_3^-]}$$

Ostwald (22) and Barth (3) showed that even at high dilutions sulphurous acid behaves as monobasic, but accurate measurement of the conductivity of extremely dilute solutions of SO_2 are very difficult, particularly at elevated temperatures where oxidation and decomposition of a complicated kind are likely to occur. It is much easier and probably more accurate to obtain the necessary value for the conductivity at infinite dilution from the values of the limiting conductivities of the separate ions. The value for the limiting conductivity of the hydrogen ion and its temperature coefficient to about 50° C. is fairly well established—Kohlrausch (12) gives it the following value:—

$$\mu_t = 315\left\{1+0.0154(t-18)-0.000033(t-18)^2\right\}$$

While this expression is only intended to be valid below 50° C. it is not probable that it is seriously in error at considerably higher temperatures.

The limiting conductivity of HSO_3^- is not so accurately known, but since it is so much smaller than that of the hydrogen ion, its exact value is of less consequence. Kerp and Baur (14) determined it as 52 at 25° C. and Lindner (15) after a consideration of the arguments considers that this is within 5% of the proper value. Accepting this value and using the formulae of Kohlrausch (13) to obtain the temperature coefficients, the following expression is derived for the limiting conductivity of the HSO_3^- ion,

$$\mu_t = 44.3\left\{1+0.0242(t-18)+0.00011(t-18)^2\right\}$$

Table IV gives the limiting conductivities of the ions calculated from the preceding formulae at ten-degree intervals from 0° to 100° C.

TABLE IV

LIMITING CONDUCTIVITIES

Temperature in ° C.	H^+	HSO_3^-	H_2SO_3
0	224.2	26.4	250.6
10	275.5	36.0	311.5
20	324.6	46.4	371.0
30	371.7	57.9	429.6
40	416.4	70.2	486.6
50	459.6	83.6	543.2
60	500.5	97.9	598.4
70	539.2	113.2	652.4
80	575.8	129.5	705.3
90	610.4	146.7	757.1
100	642.6	164.9	807.5

The concentration of the HSO_3^- ion (or the H^+ ion) is readily obtained by dividing the experimental value of the specific conductivity by the limiting value given above for the conductivity of H_2SO_3 and multiplying by 1,000 to bring the gram mols per litre. The apparent dissociation constant is then obtained from the equation.

$$K_a = \frac{[HSO_3^-]^2}{C_{SO_2} - [HSO_3^-]}$$

the values obtained being given among others in Table III.

These values for K_a are in close accord with those obtained for solutions of about the same concentrations by Lindner and by Kerp and Baur over the temperature range which they covered.

TABLE V
APPARENT DISSOCIATION CONSTANT
$K_a \times 10^3$

Temperature	0° C.	20° C.	25° C.	40° C.
Kerp and Baur	—	—	17.4	—
Lindner	31.1	19.8	17.4	11.1
Campbell and Maass	31.3	19.6	17.3	11.4

The constant varies quite rapidly with the temperature and, as shown by Lindner and by C. Maass, varies to a lesser extent with the concentration. The present work does not show the latter variation to any marked extent on account of the limited range of concentrations covered.

Since the dissocation constant does not ordinarily vary to any great extent with change of temperature it is reasonable to assume that the major portion of the variation in this case is due to the fact that an apparent dissociation constant only is dealt with here. The true constant will arise from the equilibrium.

$$H^+ + HSO_3^- \leftrightharpoons H_2SO_3$$

and will have the value

$$K^2 = \frac{[H^+]\,[HSO_3^-]}{[H_2SO_3]}$$

whereas the apparent dissociation constant obtained experimentally has the value

$$K_a = \frac{[H^+]\,[HSO_3^-]}{[H_2SO_3] + [SO_2]}$$

It is evident that K_a must always be less than K_2, but will approach it in value when K_1 is small, that is, when the equilibrium $H_2O + SO_2 \leftrightharpoons H_2SO_3$ is well toward the right as it may be expected to be at low temperatures. In accordance with this it is found that the lower the temperature the greater the value of K_a. It may, therefore, be concluded that the value of K_2, the true dissociation constant for the concentrations studied here, is at least 0.031 which is the value of the apparent dissociation constant at 0° C.

This consideration and the assumption that K_2 does not alter appreciably with temperature permits of a calculation of the maximum amount of H_2SO_3 in solution, for

$$\frac{[H_2SO_3]}{[H_2SO_3]+[SO_2]} = \frac{K_a}{K_2} = \frac{[H_2SO_3]}{C_{SO_2}-[HSO_3']}$$

so that the ratio of the amount of undissociated H_2SO_3 to the total SO_2 concentration (less that of the HSO_3' ion) is $K_a/0.031$. This would indicate that at 50° C. the fraction existing as H_2SO_3 would be only 28% of that at 0° C. At 90° C. the fraction would be 8% of that at 0° C. But at 0° C. there must still be a considerable amount of SO_2 present as such since otherwise its partial vapor pressure would be extremely low. The fractions found in this way are, therefore, *maximum* values and are probably much in excess of the actual.

That the temperature coefficient of the conductivity is considerably less than if the temperature affected the ionic mobility alone and had little effect on the ionic concentration, is an indication that the equilibrium $H_2O+SO_2 \leftrightarrows H_2SO_3$ is still playing an important role even at as low a temperature as 0° C. In the former case the change in conductivity would be about 2.4% per degree, whereas the actual value is about 1.7%.

Unfortunately the range of concentrations in these experiments is not great enough to permit of the calculation of the amount of H_2SO_3 by the method of C. Maass (16) since this depends on the change of concentration of $[H_2O]$.

The above considerations are based on the supposition that H_2SO_3 has a true dissociation constant which can be calculated according to the Ostwald dilution law, and show that this is at least as large as 0.031; there are other considerations, however, which show that it may be a considerably stronger acid than this value would indicate.

The "strong" electrolytes do not follow the Ostwald dilution law, except at extreme dilutions. According to Arrhenius (1) the strong uni-univalent electrolytes follow the dilution law up to concentrations 0.0002 molar and they all have the same dissociation constant, 0.020. In more concentrated solutions, however, the dilution law is not followed and the constant calculated in accordance with it increases rapidly with the concentration. But the fact that the dissociation constant of H_2SO_3 is at least as great as 0.031 shows that it must at least approach in magnitude that of the "strong" acids. The diminution of the dissociation "constant" of strong electrolytes with dilution is paralleled in the case of sulphurous acid. Lindner found that while the apparent dissociation constant at 0° C. was approximately 0.031 when the dilution was one or two litres the value diminished with further dilution until it was only 0.018 at a dilution of 122 litres. Since

$$\frac{K_a}{K_2} = \frac{[H_2SO_3]}{[H_2SO_3]+[SO_2]}$$

and at the greater dilution the amount of SO_2 must become very small, the only explanation would seem to be that K_2 also becomes smaller since the ratio must approach unity.

That H_2SO_3 should behave as a strong acid is not surprising in view of the fact that the organic sulphonic acids behave similarly. These may be considered as substituted sulphurous acid in that the ion HSO_3^- has become RSO_3^-. By analogy hydrogen sulphonic acid would be expected to be similar.

Assuming that H_2SO_3 is almost completely ionized, an assumption seemingly permissible in view of the above, a different conception of the question is possible. Instead of considering that SO_2 and H_2O combine to form H_2SO_3 which then dissociates into its ions, the mechanism may be considered analogous to the formation of NH_4^+ from NH_3+H^+ and the reaction written thus:

$$SO_2+OH^-+H^+ \leftrightarrows HSO_3^-+H^+$$

The extent of the reaction will depend upon the equilibrium expressed by the equations

$$[SO_2]\,[OH^-] = K_3[HSO_3^-]$$
$$\text{and } [H^+]\,[OH^-] = K_w[H_2O].$$

Combining these

$$[SO_2][OH^-] = \frac{[SO_2]\,K_w[H_2O]}{[H^+]} = K_3[HSO_3^-]$$

or
$$[H^+][HSO_3^-] = \frac{K_w}{K_3}\,[SO_2]\,[H_2O]$$
$$= K_b(C_{SO_2} - [HSO_3^-])[H_2O] \qquad (12)$$

also
$$[SO_2] = C_{SO_2} - [HSO_3^-] = Hp \qquad (13)$$

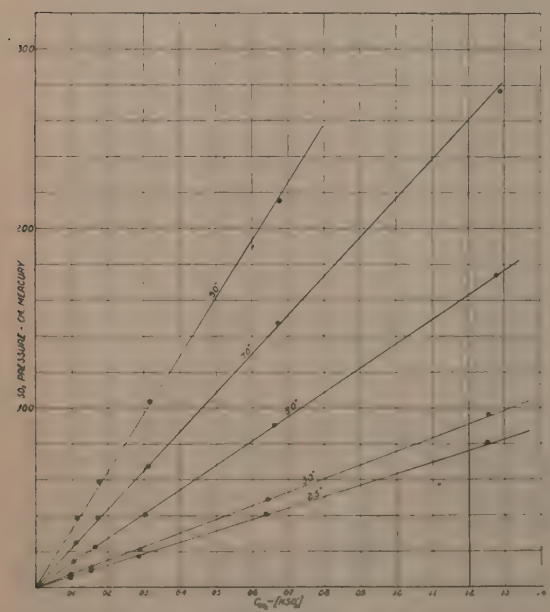

FIG. 7. *Relation of Partial Pressure of SO_2 to the concentration* $C_{SO_2} - [HSO_3^-]$.

Plotting p, the partial pressure of SO_2, against the values of $C_{SO_2} - [HSO_3^-]$ does lead to a straight line relationship as demanded by equation 13. (Fig. 7.) This correspondence does not, of course, prove that no H_2SO_3 exists as such since the range of dilution is not so great as to affect the proportionality of $[H_2SO_3]$ to $[SO_2]$ when $[H_2O]$ is constant. Even if the mechanism is correct it is quite possible that there is to some small extent a combination of the ions H^+ and HSO_3^- to form undissociated H_2SO_3. The extent of this reaction would be expected to be of the same order as that of H^+ and NO_3^- to form HNO_3 and

would be too small to be shown by the experimental evidence. In any case this method of treatment is in accord with data available at present, and is convenient to use, since only two constants are required, H and K_b, to explain the properties of SO_2 solutions as against three by the more usual method, H, K_1, K_2.

The constant K_b obtained in equation 12 is related to the apparent dissociation constant K_a through the following equations,

$$[H^+][HSO_3^-] = K_a(C_{SO_2} - [HSO_3^-])$$
$$= K_b(C_{SO_2} - [HSO_3^-])[H_2O]$$
$$K_a = K_b[H_2O]$$

Although there has been a general distinction between "strong" and "weak" electrolytes based on the degree to which they dissociate in aqueous solution, the distinction has not been as sharply drawn as might have been expected. A more definite difference between the two classes depends on the fact that the "strong" electrolytes do not obey the Ostwald dilution law, while the "weak" ones do. If, as the mechanism here proposed suggests, the few electrolytes of an intermediate type can be shown to belong to one or other of the two classes, a step has been taken toward the clearing up of the reason for the distinction. Up to the present these compounds have stood in the way since they have apparently provided a transition class and obscured the point that there is a distinction in kind rather than one of degree only.

The idea that there is a considerable proportion of SO_2 existing in the solution as such is, of course, not entirely new. Wright (27) demonstrated this by a study of the absorption spectra of the solution and of the gas. Baly and Bailey (2), by similar means, went even closer to the theory here expressed; they showed that in a solution of bisulphite there was formed a small quantity of the meta-bisulphite ion $HS_2O_5^-$. For the formation of this the SO_2 molecule must be assumed to combine with the HSO_3^- ion. Such combination would be quite to be expected if the SO_2 molecule combines with the OH^- ion.

In the introduction it was mentioned that one of the objects of this investigation was to collect data which would throw some light on the possibility of the sulphite cooking process being primarily dependent upon an "acid hydrolysis of wood." Consider a cooking liquor having a total SO_2 concentration of 0.8 molar with a base concentration of 0.4 molar, or to use the terminology of the pulp mill, 5.12% total and 1.28% combined SO_2. At 100° C. where the apparent dissociation constant is 2×10^{-3} this solution will have a hydrogen ion content of 0.002 molar. This is only one seventh of the *minimum* concentration of acid (HCl) used by Miller and Swanson (20) in a series of experiments on hydrolysis of wood "under a range of conditions comparable with those obtaining in bisulphite cooking." From this minimum the concentrations used by them increased up to 60 times the minimum. On the other hand, in actual sulphite cooking the hydrogen ion concentration is less than the figure quoted since the temperatures are higher. The conditions of their experiments are, therefore, not comparable with those obtaining in the

actual sulphite cooking process. In fact the conclusions may be drawn that the reason sulphite solutions are effective is because the sulphite addition compounds are soluble, but that the solution is so little acid under the working conditions that hydrolysis of the carbohydrates and resinification of the lignin is minimized. It is not within the field of this paper to present a theory of the sulphite cooking process, and any explanation of the mechanism of the formation of the important sulphite addition compounds will have to come from a fuller knowledge of the lignin structure.

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CHEMICAL AND PHYSICO-CHEMICAL CHANGES INDUCED IN WHEAT AND WHEAT PRODUCTS BY ELEVATED TEMPERATURES—II[1]

By W. F. Geddes[2]

Abstract

Studies were conducted on heat-treated samples of unbleached straight-grade flour milled from Western Canadian hard red spring wheat with the purpose of determining the influence of time and temperature of heating flour with normal moisture content on certain biochemical properties related to "strength". Samples were selected which showed a progressive range in baking quality including some which revealed improvement as a result of heat treatment, when baked by the basic procedure. Gluten quality was impaired in all heat-treated samples, as evidenced by decreased viscosity of leached, acidulated flour suspensions, a decreased rate and extent of imbibition of the washed out gluten, and decreased gas retention of the dough. High positive correlations between viscosity, gas retention, and loaf volume were obtained. The somewhat higher correlations between these measures of gluten quality and loaf volume when determined with potassium bromate added to the formula suggest that the bromate method gives a better measure of gluten quality than the basic procedure. Decrease in viscosity was approximately a linear function of the temperature, for constant time of heating. Ease of peptization of the flour proteins by N. magnesium sulphate, N. potassium iodide, and 5% potassium sulphate solutions showed a marked and progressive decrease with increasing severity of heat treatment. The high positive correlations between the percentage of protein extractable by these salts indicate that the magnitude of these fractions is influenced by the same inherent qualities of the flour proteins. The high positive correlations between the percentage of protein peptized by these salts and viscosity, suggest that these "qualities" are the colloidal properties of the flour proteins. High positive correlations were obtained between loaf volume and percentage of protein peptized. Increasing the moisture content of the flour, with fixed time and temperature of heating, resulted in decreased peptization indicating more extensive heat denaturation of the gluten proteins. Fractionation of the flour proteins showed that the decrease in the potassium sulphate fraction with increasing severity of heat treatment contributed to both the gliadin and glutenin fraction (as determined by the direct barium hydroxide method). Diastatic activity, as determined by the modified Rumsey method and by the rate of gas production in doughs without added sugar, revealed only slight decreases with those flours which showed improvement in baking quality due to heat treatment. Marked decreases were observed with the more severe heat treatments. Susceptibility of the starch to diastatic action was unaltered until marked damage to baking quality resulted, when significant decreases were noted. Heat inactivation of diastase evidently occurred before changes in amyloclastic susceptibility. Proteolytic activity markedly decreased with heat treatment, significant decreases being noted before diastatic activity was appreciably altered. The hydrogen ion concentration of flour extracts revealed no particular trend due to heat treatment, but the buffer value was somewhat increased. The biochemical changes investigated were, for the most part, in a direction associated with decreased baking quality, and provided no adequate explanation for the improvement in baking quality observed in certain samples when baked by the basic procedure.

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Introduction

Research in Cereal Chemistry has been directed mainly toward the determination of the physico-chemical and chemical properties of wheat flour which are responsible for strength. The various investigations have been reviewed by Sharp and Gortner (43), Bailey (5), Kent-Jones (36), and others, and need not be detailed here. It has become evident from the data accumulated after years of investigation that various factors are involved in flour strength, as defined by Humphries and Biffen (29). In a general way, these may be classified into two groups as suggested by Bailey (4),—(a) factors which contribute to the rate of gas production in dough, and (b) those which function in the retention of these gases in the fermenting dough.

The heat-treated flours, described by the author in a previous paper (19), provide an excellent opportunity for a study of physico-chemical and chemical properties related to flour strength. From one flour of given chemical composition, a series of flours have been produced which show all gradations in baking quality from excellent to very poor. Similar experimental material, with the same inherent protein, carbohydrate, enzymic and ash content, but varying baking quality, cannot be obtained in nature.

Experimental

The Biochemical studies for the most part were made on the straight-grade flours which had been heat-treated at 13.90% moisture content, for the reason that the baking quality of these flours was more extensively investigated.

The samples were selected with two objects in view—(a) to secure a progressive range in baking quality (including some samples which showed improvement on heating), and (b) to select samples which would reveal the influence of time and temperature of heating on the biochemical properties of the flour. Table I contains a list of the samples selected, together with a summary of their baking quality as determined by different methods.

(A) Studies on Wheat Flour Proteins

1. Viscosity and Imbibition

The researches of Wood (56), Wood and Hardy (57), and Upson and Calvin (51, 52) served to establish the importance of the colloidal properties of the wheat proteins. The extensive investigations of Sharp and Gortner (43) stimulated research in this field. Theoretical aspects of Sharp and Gortner's work have been discussed by Scott Blair, Watts, and Denham (42), and van der Lee (53), while Blish and Sandstedt (12), Smith (45), and others, have discussed the practical utility of the method as a measure of gluten quality. In general, the high correlation obtained by Sharp and Gortner between loaf volume and the glutenin quality factor (b) has not been borne out by subsequent investigations. More recently, Johnson (31), and Johnson and Herrington (33) have made a critical study of several variables influencing viscosity determinations.

TABLE I

SUMMARY OF BAKING QUALITY OF FLOURS SELECTED FOR CHEMICAL STUDIES AS DETERMINED BY DIFFERENT METHODS*

Treatment		Loaf volume in cc.				Single figure estimate			
Temperature in ° F.	Time in hr	Basic procedure	Basic procedure + 0.001% $KBrO_3$	Response to Br.	Basic procedure + Br. + 1.0% malt	Basic procedure	Basic +0.001 % $KBrO_3$	Response to Br.	Basic + Br. + 1.0% malt
Control		560	635	+75	672	84	99	+15	100
130	8	545	589	+44	605	87	94	+ 7	87
135	8	552	585	+33	604	87	93	+ 6	88
140	8	563	554	− 9	577	88	80	− 8	83
145	8	537	540	+ 3	533	86	87	+ 1	72
150	8	512	487	−25	496	82	77	− 1	72
155	8	500	452	−48	428	80	71	− 9	57
160	8	340	287	−53	277	53	40	−13	34
170	8	213	197	−16	213	28	20	− 8	22
160	0.5	533	611	+78	590	84	97	+13	88
160	0.75	533	551	+18	536	85	85	0	77
160	1.0	515	541	+26	542	83	84	+ 1	77
160	1.5	529	490	−39	506	83	78	− 5	68
160	2	521	486	−35	479	83	77	− 6	67
160	3	479	422	−57	404	76	66	−10	54
160	4	414	325	−87	338	67	53	−14	42
160	6	352	283	−69	306	57	41	−16	38
170	2	323	271	−52	288	52	38	−14	34
170	3	279	240	−39	279	42	29	−13	31
170	4	261	229	−32	269	39	26	−13	29
170	6	229	210	−19	247	32	22	−10	26

**Flour heat-treated for varying times and temperatures at 13.90% moisture content.*

The researches of Alsberg and Griffing (1), on the heat denaturation of gluten are of particular interest in the present study. Using the technique of Upson and Calvin (51), they observed that heating gluten at temperatures from 30 to 50° C. resulted in no impairment of swelling power, in fact the evidence indicated a slight increase. As the temperature was increased from 50° to 80° C., a progressive denaturation took place, the decrease in swelling power being a linear function of the temperature, except between 60° and 65° C. where the decrease was somewhat greater.

Kent-Jones (35), using a modified form of Ostwald viscosimeter with a relatively large bore, reported, in general, an increase in viscosity of 20% in unleached, unacidulated flour suspensions due to heat treatment. From this he considered that the increase noted in flour strength was to be ascribed to an increased imbibition of the heated proteins. No significant difference was observed between the (a) and (b) factors of Sharp and Gortner as determined on unleached, unacidulated suspensions of the flour.

Kent-Jones also reported viscosity determinations on aqueous flour suspensions incubated at 27° C. in the presence of auramine (1:10,000) to inhibit bacterial action. Viscosities were determined on the freshly made suspensions

and at intervals of 24 and 48 hr. The heat-treated flours invariably showed marked increases in viscosity with time, while the untreated flours, in general, showed a decrease (presumably due to proteolysis). These results indicate a decreased rate but increasing extent of imbibition and are suggested as an explanation for the improvement in baking quality observed by the addition of small quantities of overheated flour to ordinary flour.

Berliner and Ruter (10), repeating this work, reported that auramine did not inhibit bacterial growth, and ascribed the increase in viscosity, observed by Kent-Jones, to increased imbibition due to acids produced by bacterial development. To prevent bacterial action, these investigators made 20% flour suspensions in N/20 lactic acid and they report an increase in viscosity with time in the case of the heated flour. These results were considered a confirmation of Kent-Jones' claim that a certain degree of coagulation of the proteins by heat increases their swelling capacity.

In the studies reported in this paper, viscosity determinations of leached acidulated flour suspensions were run according to the technique outlined by Gortner (20), using, however, only one flour concentration. Since the protein content of the samples in this series is identical, the relative viscosities are in themselves an index of gluten quality. Twenty grams of flour (dry matter basis) were used for each determination, the readings being taken with a Wallace and Tiernan viscosimeter. Each sample was given the same degree of manipulation, as nearly as possible. CO_2-free distilled water at a temperature of 25° C. was used for all the determinations, which were made in duplicate, and were required to agree within 4° (W. and T).

The rate of imbibition was investigated by a modification of the Upson and Calvin procedure. Gortner and Doherty (21) utilized this method in a study of gluten washed from flours of different strengths. These authors showed that the gluten from weak and strong flours differed in regard both to the rate and extent of imbibition, the weak flour gluten dispersing much sooner and at a much lower water content than the strong flour gluten.

In the present study 50 gm flour was made into a dough with distilled water and allowed to stand under distilled water for 60 min., and the starch then washed out under a gentle stream of distilled water at 25° C. for exactly seven min. The gluten was pressed out between two glass plates to a thickness of 0.25 in. At the end of 23 min., twelve half-inch discs were cut with a cork borer and placed in distilled water at 25° C. for 60 min., and then immersed in lactic acid solution of pH = 3. Three discs were removed at intervals of 35, 70, 105 and 140 min., allowed to drain for 15 min. on an inclined, ridged glass plate, transferred to a tared moisture dish and weighed. The discs were then dried to constant weight in a vacuum oven at 100° C. and the weight of water associated with one gram of dry gluten calculated. Four determinations were made for each time interval.

Previous experiments of this kind have been conducted by weighing the wet gluten before and after immersion in acid, and expressing the imbibition as the increase in weight per gram of moist gluten. By this method, the time of

TABLE II

RESULTS OF VISCOSITY AND IMBIBITION DETERMINATIONS

INFLUENCE OF TIME AND TEMPERATURE OF HEATING STRAIGHT-GRADE FLOUR AT 13.90% MOISTURE CONTENT

Treatment		Viscosity (°W & T)	Water per gram dry gluten in grams				Rate of imbibition			Quality of gluten
Temperature in ° F.	Time in hr.		Time in min. 35	Time in min. 70	Time in min. 105	Time in min. 140	Time interval 35–70	Time interval 70–105	Time interval 105–140	
Control		182	3.74	5.44	6.37	6.93	1.70	0.93	0.56	Elastic, smooth, washed easily
130	8	173	3.51	4.83	5.86	6.64	1.32	1.03	0.78	Slightly rough; less elastic
135	8	161	3.44	4.58	5.20	6.29	1.14	0.62	1.09	Rough, more difficult to wash
140	8	154	3.55	4.62	5.51	6.16	1.07	0.89	0.65	Rough, difficult to wash, yellowish
145	8	142	3.54	4.32	5.37	6.15	0.78	1.05	0.78	Rough, difficult to wash, yellowish
150	8	130	3.34	4.53	4.69	5.60	1.19	0.16	0.91	Rough, difficult to wash, yellowish
155	8	121	3.40	4.39	4.91	5.37	0.99	0.52	0.46	Rough, very short, quite yellow
160	8	60	—	—	—	—	—	—	—	Impossible to wash out
170	8	14	—	—	—	—	—	—	—	Impossible to wash out
160	0.5	166	3.66	5.06	5.94	6.78	1.40	0.88	0.84	Smooth, elastic, washed readily
160	0.75	144	3.35	4.56	5.19	5.69	1.21	0.63	0.50	Rough, less elastic
160	1.0	150	3.65	5.34	6.30	—	1.69	0.96	—	Rough, shorter
160	1.5	138	3.69	4.88	6.17	6.69	1.19	1.29	0.52	Rough, yellowish, lacked coherence
160	2	133	—	4.66	6.05	6.82	—	1.39	0.77	Rough, yellowish, lacked coherence
160	3	105	2.98	3.91	4.14	4.21	0.93	0.23	0.07	Rough, yellowish, very difficult to wash
160	4	93	—	—	—	—	—	—	—	Impossible to wash out
160	6	67	—	—	—	—	—	—	—	Impossible to wash out
170	2	62	—	—	—	—	—	—	—	Impossible to wash out
170	3	39	—	—	—	—	—	—	—	Impossible to wash out
170	4	32	—	—	—	—	—	—	—	Impossible to wash out
170	6	19	—	—	—	—	—	—	—	Impossible to wash out

immersion in acid is limited by the necessity of removing the discs before they become soft and gelatinous, in order to avoid losses of the material incident to handling. In the method used in this paper, immersion in acid for longer times is possible since such losses would not seriously influence the results, the gluten being weighed only after its transfer to moisture dishes.

The results of viscosity and imbibition determinations are recorded in Table II.

The viscosity determinations show a progressive and marked decrease with increasing severity of heat treatment, which would indicate a definite decrease in the maximum imbibitional capacity of glutenin with increasing heat. These results are in disagreement with the data of Kent-Jones (35).

Direct determination of imbibition revealed in every case a lower imbibing capacity of the heated gluten for times up to 140 min. Marked differences in the physical properties of the gluten washed from these samples were noted. The gluten from the control washed easily and formed an elastic, smooth gluten mass. With the lower heat treatments, the gluten was less elastic, and had a characteristic rough or pebbled surface. As the heat treatment became more severe, the gluten became "shorter", yellow in color, and more difficult to wash out. With the higher temperatures the gluten particles would not adhere and were washed away with the starch. The differences in imbibition capacity recorded are probably minimum differences, since the more highly coagulated gluten particles of lowered imbibing power would undoubtedly tend to be lost during the washing process. Spousta (49), has shown that the yield of wet and dry gluten is decreased by heat treatment.

In general, a pronounced decrease in the rate of imbibition with time was observed, although the results for the 105-140 min. interval were somewhat variable. This indicates that the gluten from the heat treated samples would not attain the extent of imbibition exhibited by the control with extended time. The data of Kent-Jones (35) indicated a slower but markedly increased extent of imbibition the more severe the heat treatment. The present results are in direct disagreement with these observations. It was impossible to wash gluten from flour of normal moisture content heated at 180° F. for ten hr, but the results on flour treated at 160° F. for three hours were of interest. The imbibition rates indicated that swelling had practically ceased at 140 min.

Aside from this experimental evidence it is difficult to conceive how the gluten, contained in 0.7 gm of overheated flour even if possessed of remarkable imbibitional power when dispersed in 99.3 gm of flour, could serve to hold together the gluten of the unheated flour, and counteract its slackening "under the combined action of the proteases and acids formed in fermentation".

From the theoretical standpoint, any such increase in imbibition due to extensive coagulation is untenable. The modern view of heat coagulation of proteins as consisting of two different phases, namely denaturation of the protein, and flocculation of the denatured protein is well known and need not be detailed here. Sorenson (47) has summarized this view and points out that the process of denaturation generally involves the removal of water.

The results of the present investigation, as well as those of Alsberg and Griffing (1), are fairly conclusive evidence that heat coagulation, at least when extensive, results in a decreased capacity and rate of imbibition. Improvement in baking quality as a result of heat treatment cannot be ascribed to an increased imbibing power of the gluten.

2. Peptization of Wheat Flour Proteins

Gortner, Hoffman and Sinclair (22, 23, 24) have recently observed wide differences in the amount of protein which can be extracted from a given flour by various salt solutions. Their results showed that protein "solubility" was in reality peptization since its extent was governed by the nature of the anions and cations present in the salt solution—a typical lyotropic series of anions and cations being obtained. It was shown that these differences were not due to variations in hydrogen ion concentration.

Furthermore, with fixed concentrations of a given salt, wide differences in the percentage of total protein extractable from different wheat flours were observed. The proteins of weak flours were more readily peptized than those of strong flours, a high negative correlation between loaf volume and percentage of protein peptized by any given salt being obtained. They suggest that these differences are the result of variations in the colloidal properties of the proteins, similar differences between the ease of peptization of the gluten proteins of strong and weak flours by acids and alkalies having been previously observed.

These investigations seemed to afford another means of studying changes in the colloidal properties of flour proteins due to heat. The percentage of protein peptized by N. $MgSO_4$ and N. KI was therefore determined, using the method outlined by Gortner, Hoffman, and Sinclair (24). The results are recorded in Table III.

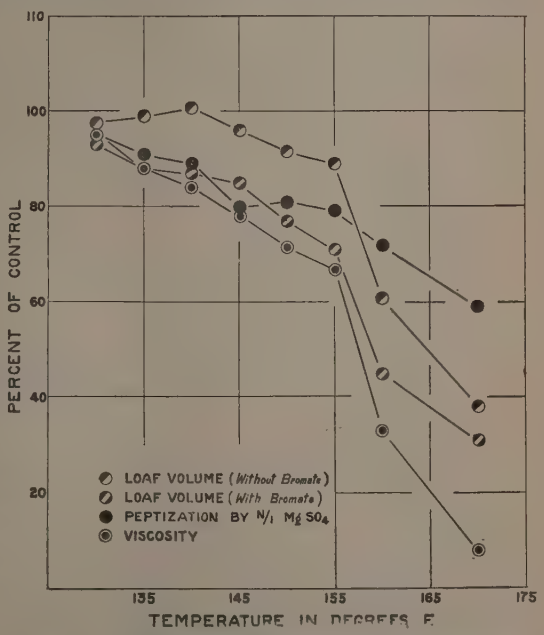

FIG. 1. *The influence of temperature of heating on viscosity, percent total protein peptized by N.$MgSO_4$, and loaf volume (with and without bromate). Results expressed as percent of control (Time of heating 8 hr, moisture content 13.90%).*

3. *The Influence of Heat on the Colloidal Properties of Flour Proteins and Their Relation to Baking Quality*

The data for viscosity, percentage of protein peptized by N. $MgSO_4$, and loaf volume (with and without bromate) have been calculated as a percentage of the corresponding value for the control sample. The results are plotted against the temperature and time of heat treatment in Fig. 1 and 2 respectively.

From Fig. 1, it may be observed that the decrease in viscosity is practically a linear function of the temperature of heating for constant time from 130° to 155° F. Above this temperature the decrease is much more rapid. These results confirm the observations of Alsberg and Griffing (1) that heat coagulation of the gluten proteins as measured by decreased viscosity, is progressive, exhibiting no definite coagulation temperature.

TABLE III

PEPTIZATION OF WHEAT FLOUR PROTEINS

INFLUENCE OF TIME AND TEMPERATURE OF HEATING (TREATED AT 13.90% MOISTURE)

Treatment		Total protein peptized in %	
Temperature in ° F.	Time in hr.	N. $MgSO_4$	N. KI
Control		18.8	61.9
130	8	17.5	61.1
135	8	17.0	60.6
140	8	16.7	59.3
145	8	15.2	58.1
150	8	15.0	56.8
155	8	14.9	55.2
160	8	13.4	52.9
170	8	11.0	47.7
160	0.5	15.8	59.6
160	0.75	15.5	58.1
160	1.0	15.7	58.0
160	1.5	15.1	57.7
160	2	15.3	58.0
160	3	14.9	56.6
160	4	14.4	55.3
160	6	13.9	53.8
170	2	13.3	53.6
170	3	12.4	52.2
170	4	12.2	51.1
170	6	11.8	49.1

The present data also substantiate the partial linear relationship between heat coagulation and temperature. Decreases in viscosity are followed very closely by decreases in percentage of protein peptized by N. $MgSO_4$ and also by loaf volume with the bromate method, the relationship between loaf volume and viscosity being particularly striking.

From Fig. 2, somewhat similar relationships are observed for increased time of heating at constant temperature, but the decrease in percentage of protein peptized by N. $MgSO_4$ does not parallel loaf volume and viscosity so closely.

As the criterion of the degree of relationship between the different measures of colloidal properties of the protein on the one hand, and loaf volume by different methods on the other, several correlations were calculated using the formula proposed by Harris (27). These are listed below:

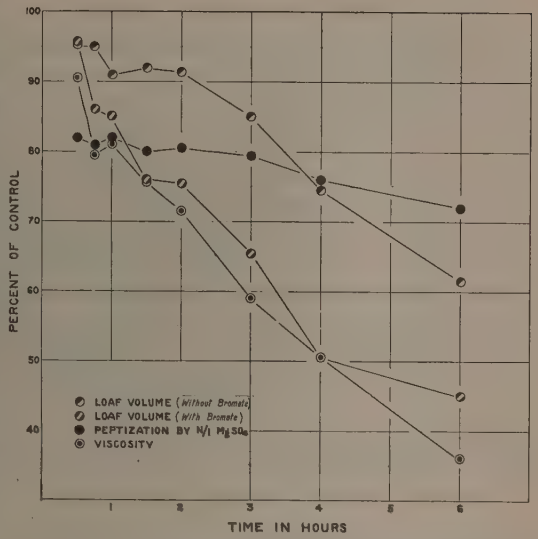

FIG. 2. *The influence of time of heating on viscosity, percent total protein peptized by N.$MgSO_4$ and loaf volume (with and without bromate). Results expressed as percent of control (Temperature of heating 160° F., moisture content 13.90%).*

I. CORRELATION BETWEEN VISCOSITY AND PERCENTAGE OF PROTEIN PEPTIZED

By N. $MgSO_4$	*By N. KI*
$r = +0.975 \pm 0.007$	$+0.942 \pm 0.017$

II. CORRELATIONS BETWEEN LOAF VOLUME AND VISCOSITY

Loaf Volume	*Viscosity*
Basic procedure	$r = +0.980 \pm 0.006$
Basic procedure + 0.001% pot. bromate	$r = +0.989 \pm 0.003$
Basic procedure + 0.001% pot. bromate + 1.0% malt	$r = +0.980 \pm 0.006$

III. CORRELATION BETWEEN LOAF VOLUME AND PEPTIZATION

Loaf Volume	*Peptization*	
	By N. $MgSO_4$	*By N. KI*
Basic procedure	$r = +0.938 \pm 0.017$	$+0.923 \pm 0.022$
Basic procedure + 0.001% pot. bromate	$r = +0.952 \pm 0.014$	$+0.924 \pm 0.022$
Basic procedure + 0.001% pot. bromate + 1.0% malt	$r = +0.957 \pm 0.012$	$+0.927 \pm 0.021$

The high positive correlations between viscosity and protein peptization is very strong evidence that differences in the extent of peptization of wheat flour proteins are the result of differences in their colloidal properties, as suggested by Gortner, Hoffman, and Sinclair (24). The higher correlation obtained with magnesium sulphate indicates that this salt is a slightly more satisfactory measure of these properties than potassium iodide. The high positive correlations between loaf volume and viscosity, and loaf volume and

protein peptized indicate that the damage to baking quality (as expressed by loaf volume) is to be ascribed chiefly to a decreased imbibitional capacity of the flour proteins. The slightly higher correlations obtained with loaf volume by the bromate method indicate that this procedure for determining baking quality is a somewhat better measure of gluten quality than the basic procedure.

The relation between loaf volume and peptization obtained in the present research differed from that published by Gortner and his co-workers in one important respect. These workers found that the larger the loaf volume, the smaller the percentage of protein peptized by a given salt solution, (resulting in high negative correlations), while in the present study high positive correlations were obtained. It must be recalled, however, that these investigators were working with native proteins, while no doubt in the present research more or less denatured proteins were dealt with. It could be expected from à priori reasoning that the more intense the heating effect, the lower would be the peptization as well as viscosity and loaf volume. Since these workers were dealing with proteins rather than proteans there would seem to be no real connection between their results and those secured in the present studies.

4. *Influence of Moisture Content of Flour on Changes in Colloidal Properties of Proteins induced by Heat.*

Since the percentage of protein peptized by N. $MgSO_4$ appeared an excellent measure of the colloidal properties of the flour proteins, this method was utilized to study the influence of different moisture contents on the extent of heat coagulation. Determinations were run on representative samples, the baking qualities of which were given in a previous paper (19). The results are recorded in Table IV.

The results, while not extensive, illustrate the very marked influence of moisture content on the extent of heat coagulation brought about by a fixed time and temperature of heating.

TABLE IV

INFLUENCE OF MOISTURE CONTENT AND TEMPERATURE OF HEATING ON PEPTIZATION OF FLOUR PROTEINS BY N. $MgSO_4$*

Temperature of heat treatment, in ° F.	Total protein peptized by N. $MgSO_4$ in %				
	Moisture content of flour at heat treatment				
	4.90%	7.25%	10.54%	13.90%	15.11%
170	—	17.2	15.2	13.3	10.7
180	—	16.2	15.8	10.0	—
200	16.4	15.5	10.6	—	—
Unheated	—	—	—	18.8	—

*Time of heating fixed at 3 hours.

The results show that an increase of approximately 3% in the moisture content has a much greater effect than an increase in temperature of 10° F. (for three hours). For example the heat coagulation brought about by heating at 180° F. with 7.25% is approximately the same as that brought about by heating flour at 4.90% moisture at 20° F. higher. The decreased peptization is again associated with decreased loaf volume.

Berliner and Ruter (10) have noted the remarkable influence of moisture content on alteration in the gluten proteins induced by heat. They observed that a sample of wheat flour previously dried in vacuo at room temperature could be heated 15 hr at 176° F. in an open vessel without damage to the gluten. Furthermore, flour without previous removal of moisture could be heated, when spread out in thin layers (to allow rapid loss of moisture) at 212°F. without any essential alteration in gluten quality.

5. *Influence of Time and Temperature of Heating on Gas-producing and Gas-retaining Capacity of Dough*

Bailey (4) suggested that: "the strength of flour is determined by the ratio between the rate of production in, and the rate of loss of carbon dioxide from, the fermenting mass of dough."

Bailey and Weigley (8) described a procedure by which such data could be obtained. The method was further developed by Bailey and Johnson (7), making it possible by a simple procedure to determine the rate of gas production in the dough as well as its gas-retaining capacity. Johnson and Bailey (32), Sherwood and Bailey (44), and Barackman and Bailey (9) used this procedure in studying the properties of fermenting dough.

This method was employed as a measure of differences (induced by heat) in the colloidal behavior of doughs. Doughs were mixed according to the basic procedure, and the dough (representing 100 gm of flour, 13.5% moisture basis) aliquoted into four equal parts by weight, and used for duplicate determinations of the rate of gas production, and loss. The determinations were made in a thermostat maintained at 30° C. (the temperature of fermentation used in the basic procedure). Readings were taken at 15 min. intervals.

Preliminary experiments on the control flour showed that the rate of gas production was practically linear for four hours, the dough reaching maximum volume in 90 min. and remaining practically constant thereafter. The duration of the experiment was therefore limited to 90 min. In instances where the duplicate results failed to agree within 6 cc., the experiments were repeated. The mean results are recorded in Table V. Typical curves constructed from this table, representing influence of temperature of heating (Fig. 3) show the results to better advantage. The rate of gas production was practically uniform for all the samples and the mean rate only is plotted.

TABLE V

INCREASE IN VOLUME OF DOUGH (B), VOLUME OF CARBON DIOXIDE LOST FROM DOUGH (C), AND SUM OF THESE VOLUMES (A) AS DETERMINED AT 15-MINUTE INTERVALS

Treatment			Volume in cc.						
Temperature in ° F.	Time in hr.		15 min.	30 min.	45 min.	60 min.	75 min.	90 min.	105 min.
Control		A	18	34	56	80	103	125	149
		B	16	31	52	74	96	106	105
		C	2	3	4	6	7	19	44
130	8	A	16	33	52	75	98	122	
		B	13	27	44	65	82	82	
		C	3	6	8	10	16	40	
135	8	A	23	37	60	79	100	124	
		B	20	33	53	72	84	84	
		C	3	4	7	7	16	40	
140	8	A	22	34	65	84	107	129	
		B	18	33	51	71	83	80	
		C	4	1	14	13	24	49	
145	8	A	14	31	53	77	100	125	
		B	12	24	38	65	80	80	
		C	2	7	15	12	20	45	
150	8	A	19	39	58	78	98	120	
		B	17	34	53	72	77	77	
		C	2	5	5	6	21	43	
155	8	A	16	36	58	82	105	126	
		B	13	32	51	67	66	67	
		C	3	4	7	15	39	59	
160	8	A	18	33	53	77	102	120	
		B	16	29	48	62	61	60	
		C	2	4	5	15	41	60	
170	8	A	12	28	47	68	93	117	
		B	11	24	37	41	44	47	
		C	1	4	10	27	49	70	
160	0.5	A	16	33	54	76	99	120	144
		B	14	29	50	72	91	102	103
		C	2	4	4	4	8	18	41
160	0.75	A	16	30	53	74	99	121	
		B	17	28	49	68	90	91	
		C		2	4	6	9	30	
160	1.0	A	13	33	51	76	98	121	
		B	12	31	47	71	88	90	
		C	1	2	4	5	10	31	
160	1.5	A	14	30	52	75	98	124	
		B	9	24	46	65	70	71	
		C	5	6	6	10	28	53	
160	2	A	17	34	55	78	104	127	
		B	15	30	49	69	71	71	
		C	2	4	6	9	33	56	
160	3	A	14	32	54	80	98	124	
		B	15	31	51	63	64	60	
		C		1	3	17	34	64	
160	4	A	14	32	53	74	96	122	
		B	12	29	48	63	62	61	
		C	2	3	5	11	34	61	
160	6	A	19	39	57	84	107	130	
		B	16	33	52	57	56	55	
		C	3	6	5	27	51	75	
170	2	A	17	35	58	81	105	128	
		B	14	30	50	56	56	55	
		C	3	5	8	25	49	73	
170	3	A	14	33	55	78	103	129	
		B	12	30	46	48	50	52	
		C	2	3	9	30	53	77	
170	4	A	14	32	54	76	98	122	
		B	11	26	38	40	43	48	
		C	3	6	16	36	55	74	
170	6	A	16	35	55	77	100	121	
		B	13	30	38	42	44	46	
		C	3	5	17	35	56	75	

The curves show that the maximum volume attained by the doughs progressively decreases with increasing severity of heat treatment, indicating lower gluten quality. Furthermore, carbon dioxide was lost in quantity sooner, these doughs attaining their maximum volume in a shorter time, indicating that the time for the first punch in commercial practice would come sooner and consequently that the total time of fermentation would be shorter. (In many commercial shops the time to the first punch in the case of straight dough constitutes about 60% of the total fermentation period). These results are in accord with the baking results which showed a decreased fermentation tolerance due to heat treatment. There is no evidence whatever of any increase in gas - retaining capacity even in the case of those samples which exhibited an improvement in baking quality. These results further confirm conclusions arrived at from viscosity and peptization studies—namely an inferior gluten quality due to heat treatment.

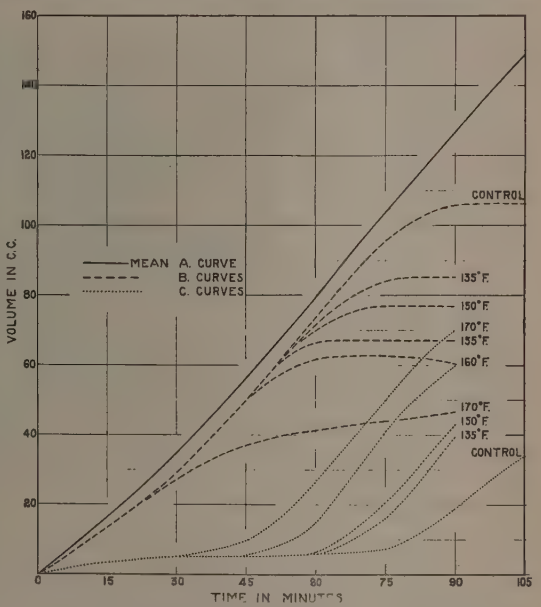

FIG. 3. *The influence of temperature of heat treatment on changes in volume which occur in systems containing fermenting doughs. Curve A represents the sum of the mean increase in volume of the dough and the volume of CO_2 lost from the dough. Curves B, the increase in volume of dough. Curves C, the volume of carbon dioxide lost from the dough.*

Simple correlations computed between loaf volume, viscosity and maximum gas retention of dough are listed below:

I. CORRELATION BETWEEN LOAF VOLUME AND MAXIMUM GAS RETENTION OF DOUGH

Loaf Volume	*Maximum gas retention*
Basic procedure	$r = +0.866 \pm 0.037$
Basic + 0.001% pot. bromate	$r = +0.947 \pm 0.015$
Basic + 0.001% pot. bromate + 1.0% malt	$r = +0.942 \pm 0.017$

II. CORRELATION BETWEEN VISCOSITY AND MAXIMUM GAS RETENTION OF DOUGH

$r = +0.920 \pm 0.022$

A higher correlation between loaf volume, with bromate added, and gluten quality as measured by the gas-retaining capacity of the dough is observed.

6. Fractionation of Wheat Flour Proteins

Fractionation of the flour proteins is attained with considerable difficulty and, in the light of our present knowledge, the usual methods employed must be considered arbitrary.

Proteins of flour are usually separated into three fractions; those soluble in 5% potassium sulphate solution, the gliadin (soluble in 70% alcohol), and the glutenin fraction. Prior to the work of Blish and his associates, glutenin was determined by indirect procedures. In "Methods of the Association of Official Agricultural Chemists (3)", the salt soluble and gliadin fractions are determined on separate portions of flour, and the difference between the sum of these fractions and the total protein is considered as glutenin.

Sharp and Gortner (43) called attention to the overlapping of solubilities observed by Bailey and Blish (6) in the solvents used, and modified the method by conducting the alcohol extraction on the residue from the extraction with salt solution (5% potassium sulphate). This procedure gave higher and what is regarded as a truer estimation of glutenin as determined by difference.

More recently, Blish and Sandstedt (12a), and Blish, Abbott and Platenius (11) developed methods for the direct estimation of glutenin, which gave results in agreement with those obtained by the Sharp and Gortner indirect method.

The more recent method, known as the "barium hydroxide method" combined accuracy with ease and simplicity of operation.

Kent-Jones (35) studied the influence of heating flour on the protein distribution as determined by the A. O. A. C. method, the Sharp and Gortner successive extraction method, and on glutenin, as determined by the Blish and Sandstedt (12a) method. He observed that "correct heat treatment" for improvement in baking quality resulted in a significant decrease in the salt soluble fraction, and a slight increase in the gliadin and glutenin fractions by the Sharp and Gortner (43) method.

Subsequent experiments were carried out, using the direct glutenin method of Blish and Sandstedt (12a), and estimating the gliadin by difference (obtained by subtracting the sum of the glutenin and potassium sulphate soluble protein from total protein). Application of this method revealed a significant and progressive decrease in the potassium sulphate fraction, but only slight alteration in the glutenin fraction until "overtreatment" was reached, when a marked decrease was observed. The decrease in these two fractions naturally resulted in an increase in the gliadin fraction as calculated by difference.

In the present study, the Sharp and Gortner successive extraction method was used, the glutenin also being determined by the "barium hydroxide" direct procedure. Preliminary trials with the former method gave low and variable results for gliadin due, presumably, to incomplete extraction by alcohol of the compact residue obtained by centrifuging the suspension in potassium sulphate solution. After a series of trials it was found that uniform and concordant results were obtained by cutting the residue into small pieces by means of a spatula, shaking for one hour, allowing to stand for six hours

with occasional shaking; this was followed by agitation in a mechanical shaker for two hours. The mean values of duplicate determinations are recorded in Table VI.

The results substantiate the observations of Kent-Jones (35) with regard to the marked decrease induced by heat in the potassium sulphate fraction. Our results, however, show an increase in glutenin by the "barium hydroxide" method. In view of the decrease reported by Kent-Jones using the Blish and Sandstedt method, determinations on a few samples were made by this procedure. The results (which are the mean of quadruplicate determinations) show no significant difference in the glutenin fraction as a result of heat treatment.

The gliadin fraction shows a fairly progressive and significant increase except in the case of the more severe treatments.

The decrease in the potassium sulphate fraction apparently contributes to both the gliadin and glutenin fractions (by barium hydroxide method). This increase in glutenin may be due to protean formation from the globulins, as proteans have, insofar as solubility is concerned, all the characteristics of a "glutenin". The results seem to emphasize the view recently expressed by Gortner, Hoffman, and Sinclair (22, 24), as to the inadequacy of our present methods of protein classification and fractionation. Their view, at least insofar as the potassium sulphate fraction is concerned, is confirmed by the results of the present work.

TABLE VI

INFLUENCE OF TIME AND TEMPERATURE OF HEATING ON PROTEIN DISTRIBUTION OF STRAIGHT-GRADE FLOUR (HEATED AT 13.90% MOISTURE)

Treatment		Protein distribution in per cent				
		Gortner's successive extraction method			Glutenin direct	
Temperature in ° F.	Time in hr.	5% K_2SO_4	70% Alcohol (Gliadin)	Glutenin by difference	Barium hydroxide method	Blish and Sandstedt method
Control		2.21	4.67	7.02	5.91	5.84
130	8	2.19	4.90	6.81	5.92	5.79
135	8	2.10	4.84	6.96	5.98	
140	8	2.08	4.87	6.95	5.99	
145	8	2.03	5.10	6.77	6.05	
150	8	2.00	5.19	6.71	6.04	
155	8	1.92	5.16	6.82	6.26	
160	8	1.74	5.27	6.89	6.75	5.74
170	8	1.41	4.73	7.76	7.29	
160	0.5	2.15	4.76	6.99	5.96	
160	0.75	2.00	4.90	7.00	5.86	
160	1.0	1.98	5.36	6.56	5.88	
160	1.5	1.96	5.33	6.61	6.11	
160	2	1.97	5.30	6.63	5.96	
160	3	1.90	5.37	6.64	6.11	
160	4	1.81	5.33	6.76	6.10	
160	6	1.79	5.41	6.70	6.69	
170	2	1.74	5.53	6.63	6.41	
170	3	1.62	5.59	6.69	6.88	
170	4	1.58	5.02	7.30	6.88	
170	6	1.48	4.94	7.48	7.25	5.81

Correlations, computed for the results of the percentage of protein peptized by N. $MgSO_4$, N. KI, and that extracted by 5% K_2SO_4, further emphasize this statement. Correlating the protein peptized by:

(a) N. $MgSO_4$ and 5% K_2SO_4 $r = +0.987 \pm 0.004$
(b) N. KI and 5% K_2SO_4 $r = +0.971 \pm 0.008$

These high positive values are convincing evidence that the magnitude of the potassium sulphate fraction depends on the inherent properties of the flour proteins as a whole and does not represent the protein belonging to any particular group.

The same characteristics of the flour proteins which influence the percentage of protein extracted by N. KI and N. $MgSO_4$ are seen to influence the percentage of protein extractable by 5% K_2SO_4 in the same direction and to the same relative extent. Their suggestion that these qualities are the colloidal properties of the flour proteins seems to be justified by the high correlations (obtained in the present research) between viscosity and peptization.

Furthermore, the results presented here, seem to extend the doubts expressed by these workers, insofar as the chemical entity of the globulin fraction is concerned, to the "gliadins" and "glutenins". It is hardly conceivable that the heating of wheat flour actually changes the amount of gliadin and glutenin, yet the percentage of gliadin shows an increase; that of glutenin shows an increase when determined by the direct "barium hydroxide" method, a decrease by the indirect method in general, and no change by the Blish and Sandstedt method.

(B) Enzymic Studies

1. Diastatic Activity and Starch Resistance

The numerous investigations concerning the relation of diastatic activity to flour strength, as well as those regarding the multiple nature of diastatic action, have been discussed in detail by Rumsey (41), Bailey (5), Malloch (38) and others and need not be detailed here. The Rumsey method for the determination of diastatic activity is affected both by the character of the substrate, and the diastase content as well as by external factors, such as hydrogen ion concentration. Sorenson (48) criticised the Rumsey method for its lack of hydrogen ion control. To meet these criticisms Malloch (37) has modified Rumsey's method by conducting the autolysis in the presence of a citrate—HCl buffer solution (pH = 4.7). With the introduction of pH control, the chief variables affecting the end result are the diastase content, and the susceptibility of the starch to diastatic action. With regard to amyloclastic susceptibility, Hermano and Rask (28), working with wheat starches prepared by the Rask and Alsberg method (40) to which diastase was added, were able to show marked differences between samples of starch obtained from different varieties of wheat and the same variety grown in different regions.

Malloch (38) has recently devised a method for determining the relative starch resistance of a series of flours. The procedure involved two determinations, (a) diastatic activity by the Rumsey method (modified by controlling the pH), and (b) starch resistance. The latter is determined by inactivating

the natural diastase of another sample of the flour (suspended in citrate—HCl buffer, pH = 4.7) by sodium tungstate, clarifying by sulphuric acid, and the inactivating agent washed out. The resulting residue is suspended in Sorenson citrate—HCl buffer solution, taka diastase added, and the suspension digested for one hour at 27° C.

The ratio between the amounts of maltose produced by autolysis, and by the addition of taka diastase is then calculated for each flour. A comparison of these ratios gives a measure of the relative diastase content, and starch resistance of the different flours.

Kent-Jones (35) reported, on the basis of diastatic activity determinations by the Rumsey method, as well as by gas measurement experiments, that there appeared to be little interference with diastatic activity, due to heating flour, until "overtreatment" was reached, when significant decreases were observed. It was also stated that "no experiments have so far revealed any perceptible alteration in the starch, when flours where heated for correct heat treatments." Berliner and Ruter (10) also report that microscopic investigations revealed no noticeable changes in the starch due to heat treatment.

In the studies reported here, diastatic activity was determined at constant pH, by the Rumsey method as modified by Malloch. As a criterion of changes in the starch, the relative "starch resistance" of the flours was determined. The method proposed by Malloch was used on a number of samples, but it proved somewhat laborious. Furthermore in the washing process incidental to removal of the inactivating agent, it was observed that starch was removed, the supernatant liquid after centrifuging giving the iodine test for starch. The use of citrate—HCl buffer solution for washing, as recommended by Malloch, was found to lessen, but not entirely to obviate, this difficulty.

In the present experiments 0.01 gm "undiluted" taka diastase (kindly supplied by Parke, Davis & Company) was added in solution to each flour suspension. Malloch used 0.03 gm taka diastase and secured smaller amounts of maltose after autolysis. Blanks on the "undiluted" taka diastase used in this work were negligible, while Malloch reported that approximately 66% of the taka diastase used by him consisted of reducing substances.

In a personal communication, Parke, Davis & Company stated that the activity of "undiluted" taka diastase is adjusted for commercial distribution by the addition of lactose. In general, two parts of lactose are added to one part of taka diastase, the percentage of lactose in the diluted preparation ranging from 60 to 70%, in order to secure the desired activity. This fact satisfactorily explains the difference observed. However, it would seem advisable to use the "undiluted" preparation in studies of this kind.

In view of the loss of starch in the washing process by the Malloch procedure, determinations of starch resistance were also conducted with the addition of 0.01 gm of taka diastase to 10 gm flour suspended in a buffer solution (pH = 4.7) by means of Rumsey's method.

Reducing sugars were measured by the procedure recommended by Quisumbing and Thomas (39), direct weighing of the cuprous oxide being resorted to. Starch resistance determined by the "added diastase" method was calculated by subtracting the milligrams of maltose, produced by autolysis of the flour without added diastase, from the milligrams obtained with the addition of taka diastase. The results are given in Table VII, and will be discussed later.

2. Proteolytic Activity

There is considerable divergence of opinion as to the importance of proteolytic activity in relation to flour strength. The investigations of Ford and Guthrie (18), Stockham (50), and Weaver and Wood (55) served to indicate the importance of proteases in flour. Recent papers by Blish and Sandstedt (13), and Cairns and Bailey (14), however, implied that proteolytic enzymes, naturally present in flour and yeast, have little influence on baking quality.

Kent-Jones (35) investigated various methods of determining proteolytic activity and concluded that the fall in viscosity of flour suspensions autolysed at 27° C. gave the most reliable results. He observed that "correct heat treatment" of flour reduced the proteolytic activity and suggested that part of the beneficial influence observed on the baking quality might be due to this cause; but the chief factor responsible for increased baking quality was believed to be an increased swelling power of the heated proteins.

Berliner and Ruter (10) reported a marked decrease in proteolytic activity due to heat treatment, as determined by the Sorenson formol titration method.

In the present study, proteolytic activity was determined by Sorenson's (46) formol titration procedure since, in an investigation of eight chemical methods, Cairns and Bailey (14) found this to be a convenient and acceptable method. The determinations were made by suspending 25 gm flour (13.5% moisture basis) in 100 cc. distilled water (saturated with toluene) and digesting in a stoppered flask for 48 hr at 37° C. with occasional shaking. The suspension was then centrifuged, filtered, and the amino nitrogen determined on a 50 cc. aliquot, using N/42 NaOH solution. Similar determinations were run on freshly made flour suspensions, and the increase in amino nitrogen due to autolysis calculated. The proteolytic activity was expressed as the increase in milligrams of amino nitrogen per 10 gm flour due to autolysis for 48 hr at 37° C. The mean results of triplicate determinations are recorded in Table VII, together with the results of the diastatic activity and starch resistance experiments.

TABLE VII

RESULTS OF DIASTATIC ACTIVITY, STARCH RESISTANCE, AND PROTEOLYTIC ACTIVITY DETERMINATIONS

Treatment		Diastatic Activity	Starch Resistance		Proteolytic activity
			"Added Diastase' method	Malloch's method	
Temperature in ° F.	Time in hr.	Anhydrous maltose per 10 gm flour in mgm			Increase in amino N per 10 gm flour, in mgm
Control		219	291	268	1.22
130	8	211	293		1.10
135	8	213	292		0.94
140	8	216	291		0.88
145	8	204	292		0.80
150	8	202	291		0.74
155	8	202	288		0.56
160	8	176			0.72
170	8	137	183	162	0.51
160	0.5	213	282	260	0.88
160	0.75	206	290	248	0.86
160	1.0	213	286	221	0.82
160	1.5	204	288	207	0.80
160	2	204	292	182	0.74
160	3	200	292	182	0.86
160	4	203	277	160	0.74
160	6	180	236	150	0.78
170	2	165	249		0.67
170	3	164	235	164	0.62
170	4	161	230		0.54
170	6	155	216		0.46

In order to facilitate comparisons, the results for each sample have been expressed as a percent of the corresponding value for the control sample (Table VIII).

These results show that wheat flour (13.90% moisture) can be heated for eight hours at temperatures up to 155° F. with no appreciable change in diastatic activity, while the proteolytic activity progressively and markedly decreases, with increasing temperature. Reference to Table I reveals that the baking quality, as determined by the basic procedure, of flours heated at 130°, 135° and 140° F. for eight hours showed improvement. These results are in agreement with observations of Kent-Jones, and are in line with the known higher thermolability of proteolytic enzymes. As the temperature of heating is increased there is a marked and progressive decrease in diastatic activity.

"Starch resistance" was determined as a convenient measure of alteration in the starch, since this property is of interest in connection with baking quality. Results obtained by the "added diastase" method show no change in this property of the starch, except for the higher heat treatments (160° F. and 170° F.), where marked decreases in baking quality were observed.

This is also in agreement with the observations of Kent-Jones (35)—that no perceptible alteration in starch occurred with "correct heat treatment" for improvement. Berliner and Ruter (10) also report no change in the starch as determined by microscopical examination.

The results show perceptible decreases in diastatic activity before the occurrence of any pronounced change in starch resistance (by the "added diastase" method), indicating that heat inactivation of diastase takes place before any alteration in starch, as measured by this method.

TABLE VIII

DIASTATIC ACTIVITY, STARCH RESISTANCE AND PROTEOLYTIC ACTIVITY*

Treatment		Diastatic activity	Starch resistance		Proteolytic activity
Temperature in ° F.	Time in hr.		"Added diastase" method	"Malloch's" method	
130	8	96	101		90
135	8	97	101		77
140	8	99	100		72
145	8	93	101		66
150	8	92	101		61
155	8	92	99		46
160	8	80			59
170	8	63	63	60	42
160	0.5	97	97	97	72
160	0.75	94	100	93	70
160	1.0	97	98	85	67
160	1.5	93	99	77	66
160	2	93	100	68	61
160	3	91	100	68	70
160	4	93	95	60	61
160	6	82	81	56	64
170	2	75	85		55
170	3	74	81	61	51
170	4	73	79		44
170	6	71	74		38

**Expressed as a percentage of the corresponding value for the control sample.*

The increase in starch resistance (or decrease in amyloclastic susceptibility) observed on extended heat treatment is surprising, and the reverse of what would have been expected in the absence of experimental evidence. Furthermore the results obtained by the two methods are not in relative agreement, although both methods show an increased resistance due to heat treatment.

The Malloch method in the present work has apparently given erroneous results, which may have been due to the writer's lack of experience with it. As a specific instance, the sample treated at 160° F. for four hours shows a diastatic activity, in Rumsey units equal to 93% of that of the control sample, while the starch resistance, as determined by the Malloch method, shows a value of 60% of that of the control. From this it must be concluded that the diastase content had markedly increased due to the heat treatment.

By the "added diastase" method the starch resistance is 95% of that of the control indicating a slight decrease in diastatic activity and increase in starch resistance due to heat treatment, which is more in line with what one would expect.

From Table VII it is observed that lower quantities of maltose were produced by the Malloch method, due to the addition of 0.01 gm taka diastase than by the "added diastase" method. While the influence of the added taka diastase on the activity of the natural wheat diastase is not known, it is believed that low results by the Malloch method may be due to two causes (a) an incomplete removal of the sodium tungstate used as an inactivating agent, and (b) loss of colloidally dispersed starch during the washing process.

The increase in starch resistance with severe heat treatment is probably to be ascribed to an alteration in the physico-chemical properties of the starch cellulose or amylopectin. In the absence of experimental evidence, a decrease in starch resistance might have been expected since it was supposed that heat treatment would cause a partial gelatinization of the starch grains. Microscopical examination, however, revealed no increase in the number of ruptured grains.

3. *Rate of Gas Production*

The series of heat-treated flours seemed excellent material for a study of the influence of diastatic activity on the rate of gas production in doughs made without added sugar. The reducing sugar content of these flours (as revealed by the blank determinations made in connection with diastatic activity) was quite uniform, hence any variation in rate of gas production must be ascribed almost wholly to differences in diastatic activity.

The apparatus and technique devised by Bailey and Johnson (7) for determining the carbon dioxide production of doughs, suggested a ready means of making the measurements. The flours were mixed into doughs using the basic formula with the exception that no sugar was added. Determinations of the rate of gas production were made at a temperature of 30° F., using a weight of dough corresponding to 40 gm of flour (13.5% moisture basis) for each determination. Burette readings were

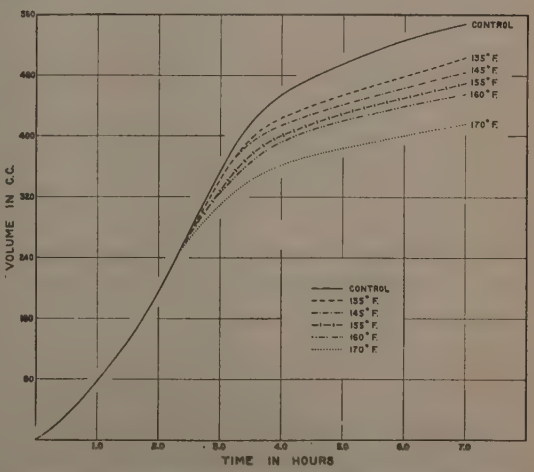

FIG. 4. *Influence of temperature of heating straight-grade flour (13.90% moisture) on rate of gas production of doughs (without added sugar) made therefrom. (Time of heating fixed at 8 hr).*

taken at 30 min. intervals for seven hours. The readings of duplicate determinations are given in Table IX. The results obtained with certain of the samples, heat-treated at varying temperatures for eight hours, are represented graphically in Fig. 4.

TABLE IX

RESULTS OF MEASUREMENTS OF GAS PRODUCTION

Treatment		Interval of time before each reading (in hours)													
Temperature in °F.	Time, in hr.	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0
		Volume of gas evolved, in cc.													
Control		25	83	134	206	274	352	424	454	476	495	511	525	538	550
130	8	23	71	125	186	255	328	390	416	434	452	465	478	490	502
135	8	34	80	132	197	269	345	401	422	439	454	467	480	492	504
140	8	34	82	133	200	273	353	400	420	436	450	462	474	485	496
145	8	34	84	136	204	275	350	393	414	429	442	455	464	475	484
150	8	29	74	128	190	260	333	384	405	420	434	446	456	466	475
155	8	29	74	127	191	258	328	379	400	415	429	440	450	460	469
160	8	31	78	131	194	266	339	376	392	407	420	429	439	448	455
170	8	34	80	131	198	269	328	346	359	374	381	391	400	409	417
160	0.5	27	76	128	195	268	340	404	423	441	456	470	482	494	503
160	0.75	29	76	126	189	258	339	387	409	424	439	452	464	475	484
160	1.0	31	78	131	196	263	336	396	421	436	450	461	473	483	494
160	1.5	34	82	135	198	269	341	400	419	435	450	460	472	482	492
160	2	32	81	129	190	260	333	388	409	425	438	450	461	471	480
160	3	33	84	137	199	274	350	394	410	425	439	450	460	470	477
160	4	27	77	126	188	260	338	385	400	415	428	440	449	456	467
160	6	23	69	109	165	232	300	362	382	398	410	421	431	441	450
170	2	32	81	128	197	268	340	369	384	396	406	417	426	434	444
170	3	24	63	107	158	223	290	344	363	377	389	400	410	418	426
170	4	26	70	115	171	236	306	347	364	378	390	400	409	419	427
170	6	33	80	125	187	255	322	349	363	376	387	397	406	415	424

The rate of gas production was fairly uniform in all samples for the first two hours of the fermentation and the mean results only were plotted for this interval. The influence of diastatic activity, however, becomes manifest at 2.5 hr, the rate of gas production being in the order of the diastatic activity of these flours. The results illustrate quite well the influence of diastatic activity in maintaining a uniformly high rate of gas production.

The coefficient of correlation between the mean rate of gas production (for seven hours) and diastatic activity was found to be:

$$r = +0.920 \pm 0.022$$

C. TITRATABLE ACIDITY, HYDROGEN ION CONCENTRATION, AND BUFFER VALUE

Somewhat divergent views are held in regard to the relation of hydrogen ion concentration and buffer value to the baking quality of flour. Investigations of Jessen-Hansen (30), the pioneer worker in this field, led him to believe that H-ion concentration was one of the main factors affecting loaf volume. Cohn and Henderson (15), and Dunlap (16), subscribe to this view. However,

the work of Grewe and Bailey (25), Fisher and Halton (17), and Halton and Fisher (26), indicates that hydrogen ion concentration is not a determining factor of great importance with regard to baking quality.

Kent-Jones (35) observed that heat treatment of wheat at 135° F. for increasing times resulted in significant increases in H-ion concentration of the flour milled therefrom, the buffer values in general also increasing somewhat. Heat treatment of several commercial flours, however, resulted, in general, in no appreciable changes in pH or buffer value. Berliner and Ruter (10) observed that flours milled from heat-treated wheats showed an increase in H-ion concentration over the unheated samples. They also observed a marked and fairly progressive decrease in titratable acidity with increasing severity of heat treatment.

It will be recalled that decreased fermentation tolerance and pronounced signs of age with increasing severity of heat treatment were observed in the investigation of the baking quality of the flours in the present study. In view of these results, it seemed of particular interest to determine the H-ion concentration and buffer value of representative samples.

Flour extracts were prepared according to the methods of the American Association of Cereal Chemists (2), and the pH determined electrometrically using the quinhydrone gold electrode. The other parts of the apparatus included a saturated calomel half-cell, a Leeds and Northrup Type C galvanometer, a Weston standard cell, an L and N potentiometer (sensitivity = 0.01 pH) and a two-volt storage battery. (Halton and Fisher (26), in a recent critical study of variables influencing the determination of H-ion concentration of flour-water mixtures, found that the quinhydrone gold electrode gave results in satisfactory agreement with those of the hydrogen electrode). In making the determinations, the H-ion concentration of a buffer solution of known pH was determined at frequent intervals as a check on the correct working of the electrode and other parts of the apparatus. The mean results of duplicate experiments (agreement required between checks being pH = 0.04) are recorded in Table X (five determinations were made on the control flour).

Titratable acidity of the flour extracts, determined by the A.A.C.C. method is also given.

In the calculation of buffer value, Van Slyke (54) suggested that buffer value be defined by the ratio $\frac{d\ B}{d\ pH}$ and that a solution has a buffer value of one when a litre will take up one gram equivalent of strong acid or alkali per unit change in pH. The buffer value over one unit in pH was calculated according to this suggestion and the result multiplied by 10^4. The tabulated values are thus equivalent to the volume in cc. of N/10 lactic acid required to change the pH through one unit over the pH range covered by the determinations.

TABLE X

INFLUENCE OF TIME AND TEMPERATURE OF HEATING ON THE TITRATABLE ACIDITY, pH, AND BUFFER VALUE OF FLOUR EXTRACTS

Treatment		Titratable acidity, as lactic acid	N/10 Lactic acid added in cc.				Buffer value
			0	1	2	3	
Temperature in ° F.	Time, in hr.		pH values				$\frac{d\ B}{d\ pH} \times 10^4$
Control		0.17	5.99	5.61	5.30	4.95	2.80
130	8	0.17	6.18	5.82	5.40	5.09	2.80
135	8	0.16					
140	8	0.15	6.19	5.85	5.44	5.13	2.83
145	8	0.14					
150	8	0.13	6.18	5.94	5.48	5.11	2.80
155	8	0.12					
160	8	0.09	6.18	5.86	5.53	5.14	2.88
170	8	0.06	6.20	5.96	5.62	5.24	3.13
160	0.5	0.15	6.13	5.85	5.46	5.07	2.83
160	0.75	0.15					
160	1.0	0.15	6.23	5.92	5.48	5.11	2.68
160	1.5	0.12	6.21	5.91	5.51	5.11	2.73
160	2	0.14	6.20	5.93	5.47	5.14	2.83
160	3	0.13					
160	4	0.10	6.16	5.89	5.48	5.12	2.88
160	6	0.09					
170	2	0.08	6.20	5.90	5.53	5.16	2.88
170	3	0.08	6.20	5.91	5.56	5.20	3.00
170	4	0.07					
170	6	0.06	6.19	5.93	5.63	5.25	3.19

The determinations were made approximately 30 days after the samples were baked, so that some change incidental to aging may have taken place. The results reveal a marked decrease in titratable acidity with increasing temperature and time of heating. The differences in pH are, on the whole, insignificant, although the values for the control sample and the half-hour treatment at 160° F. are somewhat lower. There was, however, a decided trend toward increased buffer value with increasing heat treatment. The samples were all aged for the same time, under the same conditions as regards temperature and moisture content, (which varied only within 0.6%). It is probably safe to assume that aging would have an inappreciable effect on the relative values obtained. If this assumption be valid, the results seem of interest in relation to the view held by many workers that the more highly buffered the flour, and the higher the initial pH, the longer the fermentation period required.

Fisher and Halton (17) are not in accord with this view, and point out that it is possibly based on the fact that a baker's grade or clear flour possesses greater fermentation tolerance than the corresponding patent flour, the former being also more highly buffered. The two characteristics—fermentation tolerance and high buffer value vary together and a casual relationship has been inferred. They point out that there are other and possibly more important

differences between these grades, such as quantity and quality of gluten, ash content, and constituents of the aleurone layer. In a careful study of the influence of H-ion concentration on the baking quality of a patent and a straight run flour, they found that acidification slightly increased the rate of fermentation in the case of the patent, but not with the straight-grade flour. They concluded that H-ion concentration was of little importance in breadmaking.

The results of the present investigation are interpreted as evidence in support of the conclusions reached by these workers. An increased buffer value is, in this series, associated with a markedly decreased fermentation tolerance. It is realized, however, that these flours differ in other characteristics, namely a lower diastatic and proteolytic activity, and inferior gluten quality. Baking tests with the addition of malt did not prevent the onset of the characteristic and pronounced appearance of exhaustion of the loaves; in fact, the baking quality of the heat-treated flours, determined without the addition of malt, was in most cases superior. With regard to quantity and quality of gluten, Blish and Sandstedt (13) have expressed the opinion that gluten has little or no relation to fermentation tolerance. The increase in buffer value and the decrease in proteolytic activity are, furthermore, factors commonly considered to be associated with increased stability. If these various views are provisionally accepted, it would appear that there are other important factors affecting fermentation tolerance which have as yet escaped detection and measurement.

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A COMPARISON OF THE ACTION OF B.WELCHII TOXIN WITH OTHER HAEMOTOXINS ON HUMAN AND RABBIT RED CELLS IN VITRO[1]

By J. H. Orr[2], W. A. Campbell[3] and G. B. Reed[4]

Abstract

The effect of various representative hæmotoxins on human and rabbit red blood cells *in vitro* was studied. It was found that as a result of the action of *B.welchii* toxins produced from a variety of strains of the organism a definite anisocytosis was produced and that the change in size of the cells followed a regular sequence. The first change to be noted was a development of cells having an average diameter less than the normal (microcyte stage). Further action of the toxin resulted in the replacement of these microcytes by cells having an average diameter greater than the normal (macrocyte stage). Following this macrocyte stage it was found that there was a return of the cells to a diameter very closely approximating the normal. This change in the size of the cells did not appear as a result of the action of any of the other hæmotoxins worked with viz., *B. tetani*, *V. septique*, *Strepto. scarlatinæ*, *Staph. aureus*.

1. Introduction

During the past three years a considerable amount of work has been done on the activities of *B. welchii* as the possible causative agent of pernicious anæmia. Herter (8) in 1906 first suggested a possible relationship of this organism with the disease, basing his claim on the fact that he found a greater number of *B. welchii* in stools from pernicious anæmia cases than in stools from normal cases or those suffering from other conditions. This idea was greatly extended by Cornell (2) who produced a chronic infection in rabbits as a result of subcutaneous or intrasplenic injections of cultures of *B.welchii*. The toxin of this organism was injected intravenously into monkeys by Kahn and Torry (9), and by the authors (15) into rabbits; it was found in both cases to produce a marked drop in the total red cell count of the blood with the appearance of abnormal cells suggestive of pernicious anæmia, together with a marked rise in the color index. Later Kahn and Torry (10) produced a persistent anæmia of a similar type by feeding cultures of *B.welchii* after a part of the gastric mucosa had been injured by the administration of sodium fluoride. Draper and Barach (6) also found that the injection of *B.welchii* toxin produced in rabbits a severe anæmia characterized by marked anisocytosis and polychromatophilia; but the color index was generally unaltered, or lowered. They felt, therefore, that the anæmia produced was of a secondary rather than an Addisonian type. Davidson's (5) work also threw some doubt on the ætiological significance of *B.welchii* in this type of anæmia.

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Contribution from the Department of Bacteriology, Queen's University, Kingston, Canada. The experimental work was carried out at the Lister Institute, London, England, where Dr. Campbell and Dr. Orr were guest workers.

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In an earlier investigation of the results of injections of whole cultures of *B.welchii* intra-muscularly or subcutaneously in rabbits it was found by the present authors (13) that a chronic infection could be set up which caused a marked drop in the total red cell count, a rise in the color index, and the appearance of microcytes, macrocytes, poikilocytes and nucleated red cells. The anisocytosis, expressed quantitatively in terms of the now familiar Price-Jones curves, indicated some similarity between the maximum size change curves in these animals and Price-Jones' curves for pernicious anæmia in man. The intravenous injection of toxin was also shown both by Kahn and Torry (9) and by the authors (15), to produce similar changes in the blood; the size change curves particularly were shown to be in every way similar to those produced with whole cultures and also to resemble the Price-Jones curves in pernicious anæmia. With both whole cultures and toxin the size change always followed the same sequence, a fact which is important in connection with the *in vitro* observations recorded in this paper. During the early severe stage of the anæmia, microcytes developed, and later when the count began to rise macrocytes appeared; the latter disappeared when the effect of the infection had worn off and the animal had returned to normal.

The fact that the drop in the total red cell count took place so rapidly suggested that the change was primarily one of destruction of the circulating cells. An investigation of the action *in vitro* of the toxin upon red cells was therefore undertaken. Cornell (2) recorded the occurrence of some *in vitro* anisocytosis and in a preliminary communication Reed and Orr (15) showed that contact of the Welch bacillus toxin with washed red cells *in vitro* brought about a series of changes in the red cells. The accompanying paper presents the results of a more detailed study of this problem.

Experimental Methods

In the authors' previous experiments use was made of defibrinated blood suspended in Tyrode's solution. For this series it was found that blood collected in citrated saline, washed twice in saline and made up to a 4% suspension in physiological saline was quite as satisfactory especially in view of the fact that none of the experiments lasted over four hours.

Before setting up an experiment the dilution of the toxin under investigation which would produce hæmolysis of approximately 25% of the red cells in two hours was determined. A volume of toxin was then brought to double this concentration and adjusted, when necessary, to pH 7.1–7.2. Five cc. of the toxin dilution was placed in one tube together with five cc. of a 4% blood cell suspension so that the final dilution of toxin was such that approximately one-quarter of the cells were hæmolysed in two hours, and that the final concentration of blood cell was 2%. After mixing, the reaction was determined and readjusted if necessary. In another tube was placed a broth dilution of the same concentration and reaction as in the first tube, together with the same amount of red cell suspension. This served as a control. Films for microscopic examination were made immediately after preparing the mixtures.

By experience it was found that films made at intervals of 15 to 20 minutes for the first hour, and every half-hour thereafter were sufficiently frequent to show any measurable variation in size of the red cells which might result from the action of the toxin. The films were stained by Leishman's method. At the same time that the films were made, small portions were withdrawn from each of the tubes, centrifuged, and the supernatant compared with standard tubes which contained known amounts of lysed cells.

The diameters of the cells were measured by means of a simple projection apparatus which consisted of a mirror suspended over a microscope to reflect the image to a measuring screen beside the microscope. The apparatus was set up in a dark room. The magnification was such that the normal cells had an average screen diameter of about 20 mm. Representative fields in each slide were chosen and all the cells in each field measured until the desired number had been counted.

The importance of the curves denoting variation in size of red cells in the study of anæmia may be made more apparent by referring in greater detail to their original use. Price-Jones (12) emphasized the significance of anisocytosis in pernicious anæmia by comparing quantitatively the red cell variation in normal persons with that found in pernicious anæmia patients. This he carried out by plotting, in the form of variation curves, the diameters of the cells measured as abscissæ, and the percentage of cells in each diameter class as ordinates. His curves are replotted in Fig. 1, on the scale used with the present data showing the average curve of the diameters of red cells from 20 cases of pernicious anæmia as compared with the curve from 20 normal persons. These findings were corroborated by Hampson and Shackle (7) and later by Medearis and Minot (11) who examined the blood films in two cases of pernicious anæmia both in remission and relapse and obtained, during relapse, curves similar to Price-Jones' pernicious anæmia curve, and curves closely approximating his normal curve during remission. This method of study of anæmia is now a generally used procedure. Attention will later be drawn to the similarity of some of the curves obtained in the present work to the pernicious anæmia curve of Price-Jones.

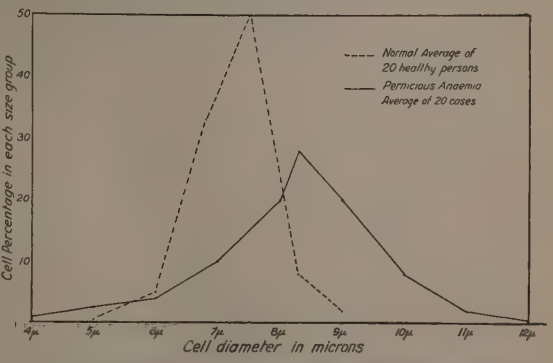

FIG. 1. *The variation in diameter in normal human red cells (500 cells from each of 20 healthy persons measured) and in pernicious anæmia cases (500 cells from each of 20 persons measured). (Data from Price-Jones (12) replotted on the same scale as used in the other figures accompanying this paper.)*

Experiments with Rabbit Red Cells

A. B. welchii toxin

Several strains of *B.welchii* were used; toxin was prepared according to Bengston's method (1) of growing the organism in a chopped beef peptone medium enriched by the addition of 0.1% glucose and incubated for 20 to 24 hours anaërobically. The supernatant mixture was then centrifuged and filtered through a Berkefeld candle. For storage the toxins were placed in 25 cc. glass bottles well-filled in order to cut down the possibility of oxidation.

The action of several samples of *B.welchii* on rabbit red cells has been studied in some detail. The results of one such experiment are given below. A sample of SR9 strain in a 1:10 dilution produced hæmolysis to the extent of some 20% of a 2% suspension of rabbit cells under the experimental conditions described earlier. The changes produced in the size of the cells are shown in Fig. 2. It may be observed that after a 15-minute action of the toxin there was a definite shifting in size to the microcyte side of the normal. After a further 15-minute period the size had shifted in the opposite direction so that macrocytes now predominated. Further action of the toxin resulted in a size variation closely approximating the normal which persisted as long as the action was followed and even after the maximum hæmolysis which the dilution of toxin was capable of producing had been reached.

The progress of the hæmolysis during the course of the experiment is indicated in Fig. 3. Up to the end of the first hour during which the experiment was in progress no definite hæmolysis had taken place which could be detected

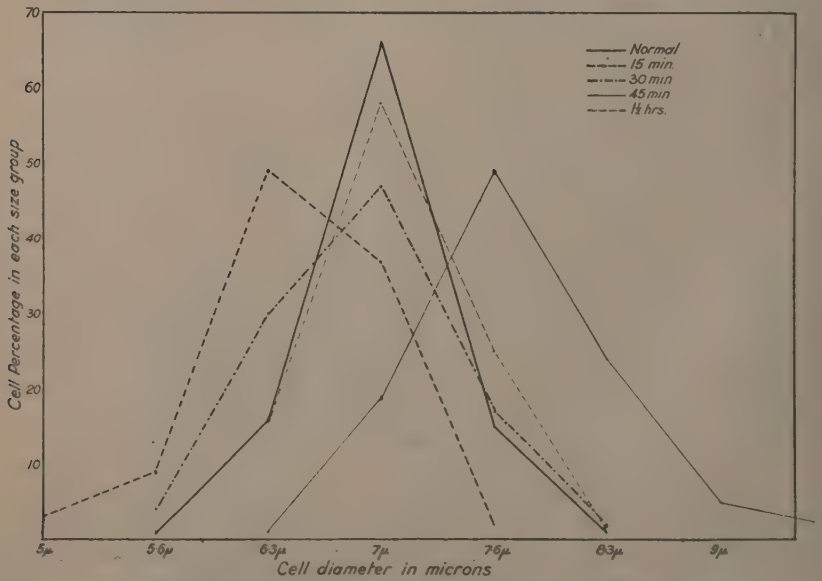

Fig. 2. *The variations in diameter of rabbit red cells as a result of the action of SR9 strain of B.welchii toxin.*

when the toxin-blood cell mixture was centrifuged and the supernatant liquid compared with the standards. Notwithstanding this fact, however, (see Fig. 2) the cells had undergone a variation in size and were practically back to

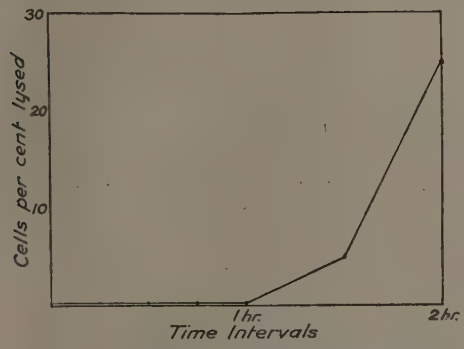

FIG. 3. *The progress of hæmolysis of rabbit red cells (2% suspension) with SR9 strain of B.welchii toxin.*

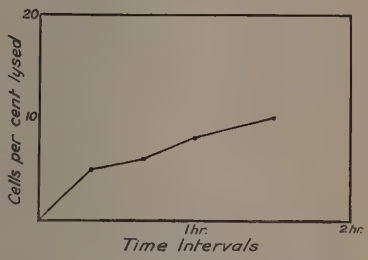

FIG. 5. *The progress of hæmolysis of rabbit red cells (2% suspension) with Tetanus toxin (3-day culture).*

normal in 45 minutes. The control tube consisting of a similar suspension of red cells and sterile broth, incubated for the same period showed no hæmolysis and no change in the size or form of the cells (Fig. 4).

Similar results have been obtained with other samples of toxin produced from this culture of *B.welchii* and other samples of rabbit red cells. More significantly, toxins produced from several other cultures of *B. welchii* have yielded the same results.

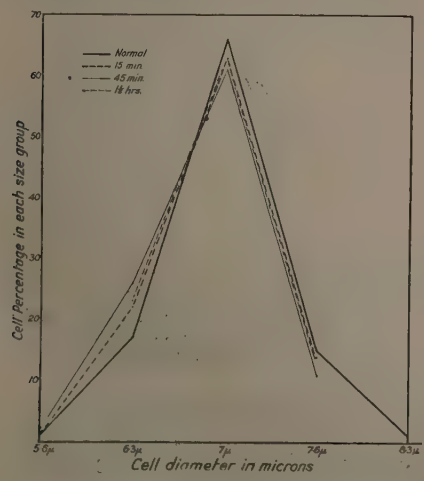

FIG. 4. *Normal rabbit red cells serving as control for the experiment represented by Fig. 2.*

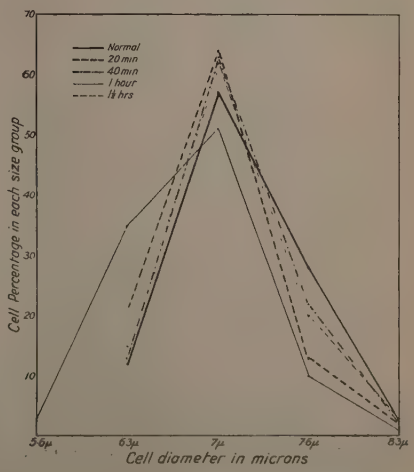

FIG. 6. *The variations in diameter of rabbit red cells as a result of the action of a Tetanus toxin (3-day culture).*

B. Hæmotoxins other than B. welchii

To determine whether or not this ability to produce an alteration in the diameter of red blood cells is a property peculiar to *B. welchii* toxin, red cells were exposed to the action of toxins of representative hæmolytic aërobes and anaërobes i.e., *V.septique*, *B.tetani*, *Strep.scarlatinæ* and *Staph. aureus*.

Tetanus toxin prepared from a three-day culture was diluted to give a degree of hæmolysis, during the period of the experiment, comparable with that produced by the *B.welchii* toxin which was used in the previous experiment. The degree of hæmolysis, shown in Fig. 5, it may be observed, is similar although slightly less than that of the *B.welchii* toxin used in the previous experiment and shown in Fig. 3. Nothwithstanding the similarity of the rate of hæmolysis in the two types of toxins the influence upon the cell size is totally different. Fig. 6 shows that there is practically no deviation from the normal cell size throughout the experiment except for a slight shoulder on the hour curve. This offers an obvious and striking contrast to the action of *B.welchii* toxin.

A sample of *V.septique* toxin was similarly diluted until its hæmolytic action was comparable with that produced by the *B.welchii* toxin. As in the case of the tetanus toxin there was practically no alteration in cell size. Fig. 7 shows a slight deviation of the 40-minute curve but this negligible size variation is also in conspicuous contrast to the *B.welchii* toxin action.

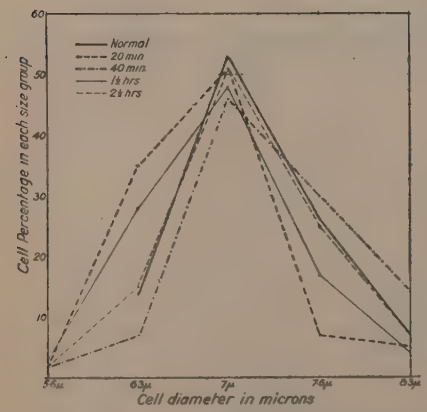

FIG. 7. *The variations in diameter of rabbit red cells as a result of the action of a V.Septique toxin.*

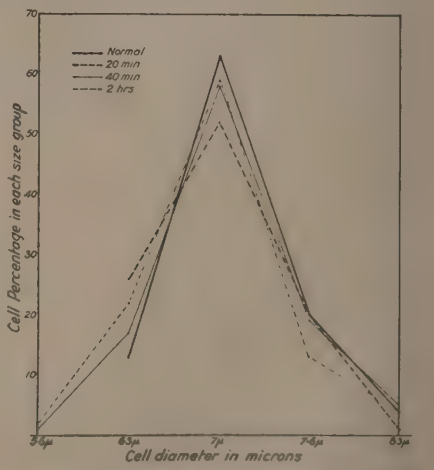

FIG. 8. *The variations in diameter of rabbit red cells as a result of the action of a Strep. scarlatinæ hæmotoxin.*

Hæmotoxins of *Staph. aureus* and *Strep.scarlatinæ* were likewise diluted until they gave a similar degree of hæmolysis. As in the case of tetanus and *V. septique* toxins, although a considerable degree of hæmolysis was produced, these toxins brought about no alteration in the size of the remaining cells. The size change, or lack of size change, produced by *Strep. scarlatinæ* toxin is illustrated in Fig. 8.

It is apparent then that *B. welchii* toxin is unique among these representative hæmotoxins of both aërobic and anaërobic bacteria. The *B.welchii* toxin produces a conspicuous variation in the size of red cells accompanying or preceding the production of hæmolysis whereas the other toxins produce hæmolysis without accompanying or preceding variation in cell size. These observations are in complete conformity with earlier *in vivo* comparisons. It has been shown by Reed, Orr and Spence (14) that the injection of tetanus toxin or streptococcus toxin into rabbits produced an anæmia without measurable anisocytosis. This result was in striking contrast with the anæmia produced by *B.welchii* toxin which was characterized by the development of very definite anisocytosis.

Experiments with Human Red Cells

Preliminary study suggested that human red cells were more sensitive to the action of toxin than rabbit cells, an observation of sufficient significance to warrant the following quantitative examination. Human blood was collected in citrated saline and treated in exactly the same manner as the rabbit blood.

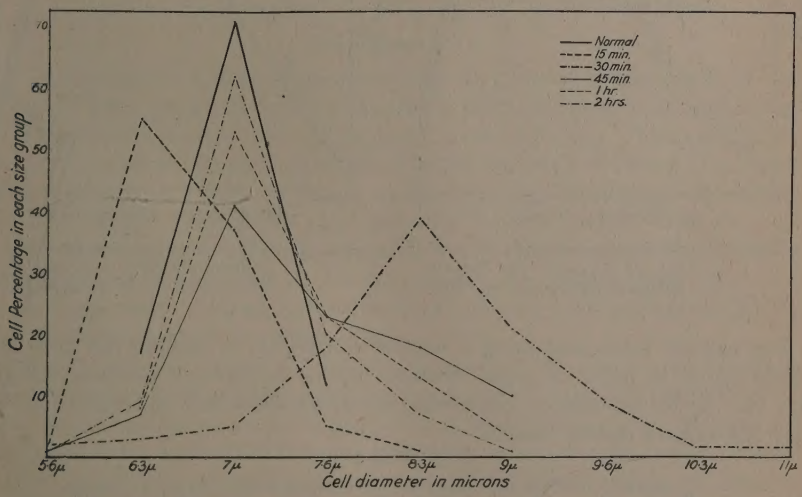

Fig. 9. *The variations in diameter of human red cells as a result of the action of SR9 strain of B.welchii toxin.*

A. B.welchii toxin

A sample of the SR9 toxin used in the first mentioned experiment with rabbit cells, was brought into contact with human red cells: the extent of the size change produced is shown in Fig. 9. After the toxin had been acting for 15 minutes there was a definite development of microcytes similar to that which occurred in the case of the rabbit cells, while after 30 minutes there was a similar and much more definite shift in size toward macrocytes than was found with the rabbit cells (Fig. 2). Following this macrocytic stage, just as

in the case of the rabbit blood, the cells returned gradually toward their normal size. The modes and standard deviations of these curves illustrating variations in size are given in Table I.

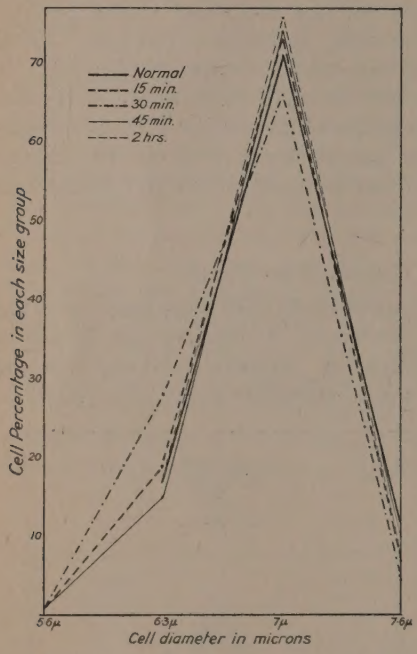

FIG. 10. *Normal human red cells serving as a control for the experiment represented by Fig. 9.*

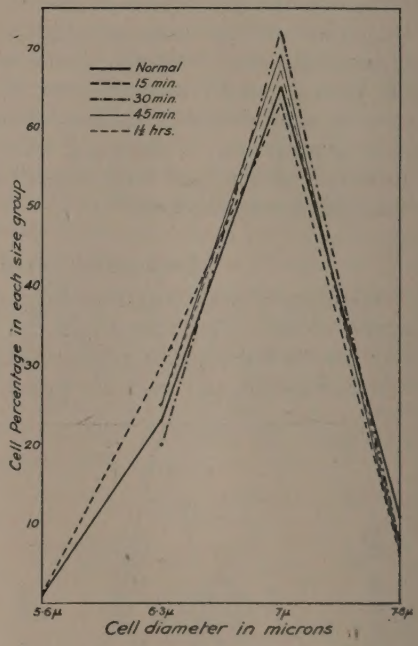

FIG. 13. *The variations in diameter of human red cells as a result of the action of a V. septique toxin.*

The control tube containing a similar suspension of human red cells and sterile broth in place of toxin, showed neither hæmolysis nor size change, Fig. 10. Other samples of toxin made from this and other strain of *B.welchii* were tested with similar results.

TABLE I

ACTION OF *B.welchii* TOXIN ON HUMAN RED CELLS, *in vitro*
MODES AND STANDARD DEVIATIONS CALCULATED FROM THE CURVES

Samples	Curve shown in Fig.	Mode	Standard Deviation
Cells *in vitro* (no toxin)	9	7	.38
Cells *in vitro*—toxin 15 min.	9	6.6	.47
Cells *in vitro*—toxin 30 min.	9	8.5	1.00
Cells *in vitro*—toxin 45 min.	9	7.6	.80
Cells *in vitro*—toxin 1 hr.	9	7.4	.65
Cells *in vitro*—toxin 2 hr.	9	7.2	.56
Price-Jones normal cells	1	7.1	.60
Price-Jones pernicious anæmia cells	1	7.9	1.25

B. Hæmotoxins other than B.welchii

V.septique toxin was diluted until it gave an hæmolytic action similar to that of the *B.welchii* toxin (Fig. 12). The influence of this toxin on the size of human cells is shown in Fig. 13. It is apparent, as in the case of rabbit red cells, that no size change is produced. *Staph. aureus* toxin diluted to produce comparable hæmolysis also failed to effect a size change in human red cells.

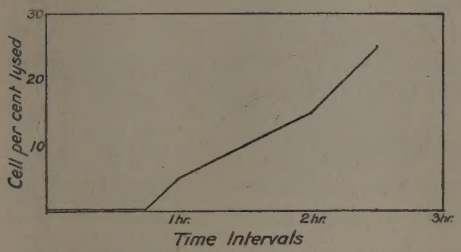

FIG. 11. *The progress of hæmolysis of human red cells (2% suspension) with SR9 strain of B.welchii toxin*

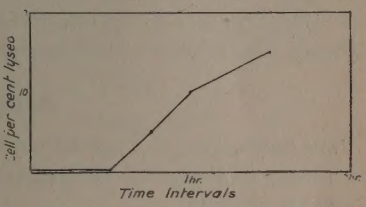

FIG. 12. *The progress of hæmolysis of human red cells (2% suspension) with V. septique toxin.*

Discussion

The size variation curves have shown that as a result of the action of the toxin of different strains of *B.welchii* a definite size change has been undergone by red blood cells, both rabbit and human, in the test tube. This change, or rather this series of changes, follows a definite sequence. There is to begin with a production of cells having a smaller average diameter than normal (microcyte stage). Following this the cells become larger, their average diameter becoming appreciably greater than normal (macrocyte stage), and this in turn is followed by a return to the normal size. The results obtained in this series of experiments suggest that human red cells are more susceptible to the action of the toxin than are rabbit cells.

Attention may be drawn to the resemblance of all the curves denoting variation in size of red cells produced by the action of *B.welchii* toxin *in vitro*, especially the maximum variation from the normal, to the curve obtained by Price-Jones, from films taken from pernicious anæmia cases. This resemblance is particularly pronounced in the case of the 30-minute curve showing the action of *B. welchii* toxin on human blood (Fig. 9). The mode here as with the Price-Jones curve is definitely on the macrocyte side of the normal, and the range of size in both cases is similarly wide. This similarity is perhaps more precisely perceived by comparing (in Table I) the calculated modes and standard deviations of the *in vitro* variation curves of Fig. 9, and those of the Price-Jones curves of Fig. 1. It is apparent that the Price-Jones curve for normal cells shows a similar mode but a slightly greater standard deviation than the present experimental cells. However, the curve for pernicious anæmia cells shows an increase in the mode and in the standard deviation similar to those of the curves obtained from the influence of *B.welchii* toxin, *in vitro*.

It seems a matter of some significance, that when human blood in a test tube is acted on by this toxin, a change is produced in the size of the cells which is so similar to that obtained from actual cases of pernicious anæmia in man, especially if the contention of Price-Jones is accepted, that this anisocytosis is the most precise characteristic of pernicious anæmia.

Another point which is to be dealt with at greater length in a subsequent paper is the development of this characteristic size change before any hæmolysis develops. The repeated occurrence of this phenomenon suggested the possibility that the size change might be due to a reaction of the hæmotoxin entirely different from that which is responsible for the actual lysis of the cells.

Acknowledgment

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